SENTINELS OF ARCTIC ECOSYSTEM HEALTH: POLAR BEAR AND ARCTIC FOX

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SENTINELS OF ARCTIC ECOSYSTEM HEALTH: POLAR BEAR AND ARCTIC FOX

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ABSTRACT

Climate change is impacting human, wildlife, and ecosystem health in the Arctic. Currently, we lack sufficient information to fully appreciate the ramifications of these changes and are thus ill equipped for predicting, mitigating or adapting to the outcome of such impacts. Warming in the Arctic has generated a need for baseline information on biodiversity and ecosystem health such that change over time may be assessed. Sentinel species can be used to monitor and therefore, intervene to prevent adverse health outcomes before they manifest at the population level. This dissertation examines the use of polar bears (*Ursus maritimus*) and arctic foxes (*Alopex lagopus*) as sentinels for climate change in the Arctic. To this end we: develop hematological biomarkers in polar bears which can be used to model change over time in health; demonstrate relationships between this biomarker and infectious agent exposure (e.g serology); and establish prevalence and risk factors for infectious agents that can serve as indicators of change in disease occurrence at the Arctic marine-terrestrial interface. We found that den emergent female polar bears with dependent young were the most immunologically vulnerable cohort and suggest therefore, that they be targeted in future monitoring efforts. We also detected evidence suggesting serologic exposure of polar bears to morbillivirus and *Toxoplasma gondii* may be associated with immunological status and age (morbillivirus only). Furthermore, we used molecular epidemiologic techniques to identify the strain of the highly lethal morbillivirus in arctic fox as "arctic" canine distemper virus and the species of *Echinococcus* in arctic fox on the Alaska North Slope as *Echinococcus*

multilocularis. The results of this study illustrate the utility of the "One Health" approach in addressing the impacts of climate change. Understanding Arctic ecosystem health will require the collaborative efforts of experts in diverse fields as well as input from local, traditional ecological knowledge over the proper spatial and temporal scales.

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LIST OF ABBREVIATIONS

A	adenine
ACIA	Arctic Climate Impact Assessment
AE	alveolar echinococcus
ASVL	Alaska State Virology Laboratory
AVPS	Alaska Veterinary Pathology Services
bp	base pair
BLAST	Basic Local Alignment Search Tool
C	cytosine
CDV	canine distemper virus
CE	cystic echinococcus
CI	confidence interval
CMV	cetacean morbillivirus
CSU	Colorado State University
DEW	Defense Early Warning
DMV	dolphin morbillivirus
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
F	fusion
G	guanine
GTR	generalized time reversal

H	hemagglutinin
HKY	Hasegawa-Kishono-Yano
IACUC	Institutional Animal Care and Use Committee
IFA	indirect immunofluorescence assay
IgG	Immunoglobulin G
IHC	immunohistochemistry
INBRE	IDeA Networks for Biomedical Research Excellence
IPCC	Intergovernmental Panel on Climate Change
ISIS	International Species Information System
LL	lower limit on 95% confidence interval
M	molar
MD	% change in count means
mg	milligram
$MgCl_2$	magnesium chloride
ML	maximum likelihood
ml	milliliter
mM	millimolar
nad1	nicotinamide adenine dinucleotide dehydrogenase 1
NCBI	National Center for Biotechnology Information
NCCR	National Center for Research Resources
NA	not applicable
ND	not determined

NIH	National Institutes of Health
NNI	nearest neighbor interaction
NPRA	National Petroleum Reserve-Alaska
OADDL	Oklahoma Animal Disease Diagnostic Laborat
OC	organochlorine
OR	odd's ratio
P	phosphoprotein
PAUP	Phylogenetic Analysis Using Parsimony
PBS-EDTA	phosphate buffered saline-ethylene diamine tetra acetic acid
PCR	polymerase chain reaction
PDV	phocine distemper virus
PMV	porpoise morbillivirus
PHYML	Phylogenetic Inferences Using Maximum Likelihood
PIT	passive integrated transponder
r	Pearson product-moment correlation coefficient
RNA	ribonucleic acid
rpm	revolutions per minute
RT-PCR	reverse transcription polymerase chain reaction
SD	standard deviation
SE	standard error
SBS	southern Beaufort Sea
SN	serum neutralization

SPR	subtree pruning regrafting
T	thymine
TN	Tamura-Nei
UAF	University of Alaska Fairbanks
UGA	University of Georgia Athens
UL	upper limit on 95% confidence interval
USGS	U. S. Geological Survey
WADDL	Washington Animal Disease Diagnostic Laboratory
x g	times gravity
µl	microliter
µm	micromolar
%	percent
°C	degrees centigrade
χ^2	Chi square

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INTRODUCTION

The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2007) determined warming of the climate system to be unequivocal. Projections for the 21st century demonstrate that global warming will accelerate with predictions of the average increase in global temperature ranging from 1.8° C to 4°C. In addition to a rise in temperature, IPCC models also predict an increase in precipitation, especially during the winter months. Large-scale changes in ocean currents and pH are anticipated with warming of the climate system and increases in CO_2, with large scale food web consequences predicted. In the past 100 years, average Arctic temperatures have increased at almost twice the global rate (IPCC, 2007). Over the last thirty years, seasonal minimal sea ice extent has decreased by 45,000 km^2/year (Parkinson and Cavalieri, 2008). Coastal and marine ecosystems, in particular, are anticipated to be impacted by increasing temperatures, changes in precipitation patterns, sea-level rise, altered salinity, ocean acidification, and more frequent and intense weather events (Portier et al., 2010). These changes are expected to have both direct and indirect impacts on ocean and coastal ecosystems by affecting community structure, biodiversity, and the growth, survival, persistence, distribution, transmission, and severity of infectious agents, vectors and reservoirs (Niemi et al., 2004).

Concerns for Climate Change Impacts on Human Health

Climate change was reported as the most significant global health threat of the 21st

century (Lancet and University College London Institute for Global Health Commission; Costello et al., 2009). The Commission statement stressed the necessity of an integrated multidisciplinary approach for understanding and potentially reducing the adverse health effects upon humans resulting from climate change-based responses. Furthermore, they emphasized that climate change will have the greatest impact on those who are already the poorest in the world, and increase existing inequities and health disparities (Costello et al., 2009). Poverty increases vulnerability to the health effects of climate change, due largely to inadequate access to health care alone. It also increases a population's risk of displacement by extreme weather events or environmental degradation. This is due to the fact that these groups are not well equipped to recover easily, and as a result, may suffer much higher disease risks. Those who work outdoors and people living in coastal and riverine zones have also been identified as "at risk" to groups to climate-induced environmental changes resulting from flooding and extreme weather events (Portier et al., 2010). Climate is directly linked to health through changing patterns of disease and mortality, extreme weather events, and security of food, water, and shelter. Resident indigenous populations of the Arctic are especially vulnerable to climate change due to their close relationship with and dependence on the land, sea, and natural resources for their well being (Parkinson and Berner, 2009), socioeconomic status, and limited access to healthcare infrastructure in some remote regions. In order to develop effective adaptation strategies to mitigate risks posed, we must understand the local needs of individual populations. Climate change adaptation strategies should include measures to provide early warning in order to reduce exposure and susceptibility to climate change

associated health hazards (Portier et al., 2010). Two of the health concerns for peoples of the Arctic with climate change focused upon in these studies are those relating to food security (e.g. nutrition and increasing physical hazards associated with subsistence) and changing patterns of disease exposure.

Climate Change Impact on Arctic Marine Food Web Dynamics

Over the last few decades, the extent of annual sea-ice has decreased by 5-10% and average thickness by 10-15%. Sea-ice is also forming later and breaking up earlier than in previous decades (Post et al., 2009). Sea-ice is critical for current polar marine ecosystems. For example, it provides a habitat for photosynthetic algae and nursery ground for invertebrates and fish during times when the water column cannot support phytoplankton growth. When the ice melts, organisms are released into the surface water, cultivating large ice-edge blooms important to the overall productivity of the seas (Michel et al., 2002). Ice algae are an essential part of the marine food web, contributing an average of 57% to the total arctic marine primary production (Gosselin et al., 1997). Sea-ice dynamics affect various marine populations and the overall food web in polar waters (the cryopelagic food web), which has provided the foundation for the sustenance for generations of Inuit peoples. In more practical terms, the sea ice is a needed platform for hunting and moving around the Arctic for many species, including humans.

Declines in arctic marine biodiversity expected with climate change may affect availability of traditional foods thus presenting an issue for food security. There is

evidence that changes in biodiversity in the marine food web are already occurring. For example, phytoplankton have been declining globally over the 20th century, and long term population decreases were negatively correlated with rising sea surface temperatures and changing oceanographic conditions (Siegel and Franz, 2010). Phytoplankton forms the basis of the marine food chain and sustains diverse assemblages of species ranging from tiny zooplankton to large marine mammals, seabirds, and fish. Most of the phytoplankton declines occurred in polar and tropical regions in the open oceans where most of the phytoplankton production occurs. Furthermore, an analysis of global biodiversity patterns for over 11,000 marine species, ranging from zooplankton to sharks and whales, found striking similarities among the distribution patterns, with temperature strongly linked to biodiversity (Tittensor et al., 2010). These results imply that future changes in ocean temperature may greatly affect the distribution of biota in the marine environment, and thus alter pathways for environmental agents of disease, such as infectious and toxic agents.

Climate Change and Security of Traditional Foods in the Arctic

The availability and cost of store-bought foods are prohibitive in small, isolated communities with high poverty rates that are based on combinations of cash and subsistence economies. Shipping costs for goods (such as groceries) to these isolated communities has increased with the rise of fuel and postage costs. As a result of high food costs and socioeconomic factors, subsistence foods provide food security for families in northern communities. Declines in Arctic biodiversity may affect availability

of traditional foods. Subsistence foods provide between 24% and 98% of energy, protein, omega-3 fatty acids, iron, and vitamins A and B^{12} in the diet of Alaska Natives (Verbrugge and Middaugh, 2004). The replacement of subsistence foods that are low in fat and high in omega-3s, with a market-based Western diet has increased the risk of cardiovascular disease and diabetes in Alaska Natives (Verbrugge and Middaugh, 2004). Decreased access to traditional foods due to climate change may exacerbate this problem. Climate change is already impacting northern communities. For example, the weakening and retreat of sea-ice has made access difficult and hazardous for subsistence hunters in some regions. Hunting has already become more dangerous and less successful, with greater occurrence of injuries and death by drowning (A. Parkinson, 2010 pers. comm.). Declines in biodiversity coupled with unpredictability of sea-ice will adversely affect the ability of northern peoples to sustain traditional ways of life and also the well-being of other apex predators of the ecosystem such as polar bears and arctic foxes.

Climate Change Impact on Biodiversity and Infectious Disease in the Arctic

Predicted effects of climate change on biodiversity include: changes in distribution, geographical range, and abundance of species (including invasive species). These changes will impact genetic diversity as well as behavior of migratory species, and in severe cases result in local extirpations (e.g., rats on sea bird colonized islands, competition between red fox and arctic fox). Alteration in migration routes of prey and subsistence species not only impacts nutrition, but also has significant implications for disease exposure. For example, as species change ranges carrying potential pathogens

with them, new exposures may occur in naïve hosts (e.g., unaffected carrier introduces pathogen to a region where highly vulnerable hosts are impacted). In some regions new diseases may be established and alteration in host feeding ecology may exacerbate these effects. In addition, climate change may increase the transmission cycle of vectors (e.g., magnitude related to abundance of vectors, duration of effective disease transmission, timing with hosts and parasites) as well as the range and prevalence of vectors and reservoir species. Alteration in parasite ecology in the Arctic, associated with warming trends, has already been observed. For example, Kutz, et al. (2005) documented a shortened life cycle of the nematode parasite (*Umingmakstrongylus pallikuukensis*) of muskoxen, and a resulting increase in parasite burdens within the hosts. This increase has been suggested as a contributing factor to increased mortality of muskoxen in Norway (Ytrehus et al., 2008). In addition, the increased occurrence of the filarioid nematode *Setaria tundra* in Finnish reindeer (*Rangifer tarandus*) has recently been linked to higher temperatures (Laaksonen et al., 2010).

Climate plays a large role in governing population dynamics of arvicoline rodents. These species are not only an important prey base for some Arctic predators, but also hosts for some zoonotic diseases. Throughout 2006-2007 there was a sudden, large outbreak of a form of hemorrhagic fever called nephropathia epidemica, caused by the Puumala hantavirus in northern Sweden. The affected region had experienced a record-breaking warm winter. The lack of snow cover likely resulted in a greater contact between the bank vole (*Myodes glareolus*) reservoirs and humans, as voles concentrated inside or

nearby human dwellings (Evander and Ahlm, 2009). There is concern that climate will alter the ecology of *Echinococcus* spp., an important parasitic zoonosis in the Arctic, leading to increased infection pressure to humans. Alterations in fox and rodent population dynamics may also impact the ecology of Arctic rabies, a well-known lethal zoonosis.

Climate change will affect the microbial population of the macro-environment (soil, air and water), as well as host and vector populations. The melting of permafrost, for example, can compromise the integrity of sewage lagoons, allowing the discharge of waste into rivers, oceans and into contact with human and wildlife hosts. An increase in flooding events may result in outbreaks of waterborne infection, such as *Giardia* spp. or *Cryptosporidium* spp.

Climate alteration is also expected to impact the long-range transport and fate of contaminants in the Arctic. Persistent organic pollutants (POPs) volatilize from lower latitudes and are transported to the Arctic where they are then deposited via precipitation and through other mechanisms (Lohmann et al., 2007). Locally, melting of glaciers may directly increase contaminant levels entering the food chain as contaminants deposited during their years of historical use are liberated (Blais et al., 2001). Permafrost has acted as a sink for POPs, and as it melts the contaminants are released (Suk et al., 2004). Reduced marine ice cover will increase ocean-atmosphere gas exchange and therefore, deposition of contaminants like polychlorinated biphenyls (PCBs) and toxaphene, which

are still entering the Arctic Ocean via the atmosphere (AMAP, 2003). Indirect effects may occur as predators shift prey species. For example, increasing contaminant loads have been documented in some polar bear sub-populations, possibly as a result of dietary shifts due to declining sea-ice (McKinney et al., 2009). Stressors including decreasing access to optimal prey species, heat stress, exposure to increasing levels of contaminants and increasing burdens of infectious agents may act in synergism to decrease fitness of arctic apex predators who reside at the top of the arctic marine food web.

Climate Change and Ecosystem Health: The "One Health" Approach

Anticipated changes in the Arctic have generated concern for the health of both human and wildlife populations. The health of ecosystems is reflected in the health of animals inhabiting those ecosystems; and the animals affect the health of the system as well. Viewing the health of animals and humans as intertwined ("One Health") can allow the development of indicators that are suitable for both. The term 'health' has historically been used to describe the vitality of individuals and, more recently, of populations (humans, domesticated animals and wildlife). The extension of the use of the term to describe regional ecosystems is a response to accumulating evidence that human-dominated ecosystems have become highly dysfunctional (Vitousek et al., 1997). The concept of ecosystem health integrates the health humans with that of animals and ecosystems. Here we use the term ecosystem health to indicate an ecosystem's resilience, biodiversity, and ability to provide services to humans (Rapport et al., 1998). Rapport et al. (1998) define indicators of ecosystem health as: vigor measured in terms of 'activity,

metabolism or primary productivity'; organization which can be assessed as the diversity and number of interactions between system components; and resilience (counteractive capacity) measured in terms of a system's capacity to maintain structure and function in the presence of stress.

There is a need for greater data sharing and collaboration between animal and human health professionals regarding animal and human populations. In the United States, links between human and animal disease surveillance are still limited by the separation of human and animal disease monitoring efforts. With the exception of disease-specific programs for rabies and potential bioterrorism agents, there are few mandates for direct communication between animal and human health officials. A survey of United States Veterinarians in agriculture departments in 43 states found that only 19% of states require reporting of zoonotic diseases to public health departments (Rabinowitz et al., 2009). The increased use of molecular techniques to understand the evolution of pathogens crossing between animal and human populations and between species has been identified as a useful means to address knowledge gaps in linking animal and human health (Rabinowitz et al., 2009). The increasing sophistication of informatics tools and surveillance using molecular epidemiologic approaches can greatly enhance our ability to monitor ecological health. Enhanced animal surveillance and detection of emerging (and re-emerging) zoonoses are needed to assess ongoing challenges posed to ecosystem health.

Using Sentinels to Assess Arctic Ecosystem Health

The precise consequences of changes in climate in the Arctic cannot be predicted adequately based on our current understanding of these systems, and certainly not via an assessment of the status of a single species (e.g., humans). This deficiency of information is due to a number of challenges including but not limited to: the lack of knowledge of the natural history of many organisms in the Arctic; logistical challenges associated with collecting data in the Arctic environment, and inadequate surveillance and diagnostics. In the sea-ice ecosystem, the study of individual ecological components is unfeasible and inefficient. The dearth of information concerning impacts of ongoing changes in the Arctic and the difficulty of gathering information in these remote ice-covered areas underscores the need to identify and monitor sentinel species.

A sentinel species is defined as one that can provide insight into the health of an ecosystem, based on user-defined (e.g., researchers, conservationists or policymakers) objectives (e.g., disease, parasites, toxics, climate change, habitat destruction), coupled with the utility and vulnerability of this species to the stress (Aguirre, 2009). Because animals typically have shorter, more physiologically compressed life-spans than humans, the latency periods for the development of some diseases are shorter. Animal studies are also free from some confounders (lifestyle and occupational risk factors) that complicate interpretation of human studies (Backer et al., 2001).

Use of animals as sentinels for human health threats dates at least as far as the era when

coal miners brought canaries into mines to provide warning of toxic gases. Sentinel herds of livestock are utilized to indicate exposure to brucellosis and other transmissible diseases (Rabinowitz et al., 2009). In the 1962 publication the Silent Spring, Rachel Carson wrote "our fate is connected with the animals". Carson recognized that humans and animals share local environments, air, water, and food chains and that the molecular, biochemical, and cellular processes, which respond to toxic agents are common among most vertebrate species. This work launched the modern environmental movement. Ecosystem approaches to health, or EcoHealth emerged in the 1990s, and include the "One Health" initiative and Conservation Medicine. These movements emphasize the links between human, animal and ecosystem health and support the collaboration of practitioners and researchers in ecology, wildlife biology and management, fisheries, toxicology, human medicine and veterinary medicine. Studies utilizing the ecosystem health approach differ from traditional, single discipline studies in that they require the expertise of professionals from diverse disciplines to work together. This is clearly reflected in the diverse backgrounds of the Graduate Committee that guided the following body of research for this PhD student.

Species with limited distributions or with specialized adaptations that depend on sea-ice will be impacted by climate change in the Arctic. Polar bears, for example, require sea-ice for prey access and other aspects of their life history (Stirling and Lunn, 1997; Amstrup, 2003). Observed declines in sea-ice availability associated with reduced body condition have been linked to declines in reproduction, survival, and population size in

some subpopulations. Continued habitat reduction is likely to increase nutritional stress among polar bears. Observed (Comiso, 2006) and projected (Holland et al., 2006) sea-ice declines led to the conclusion the future welfare of polar bears may be diminished (Amstrup et al., 2008) and to their listing as a threatened species in the US (*U.S. Fish and Wildlife Service*, 2008).

As apex predators in the Arctic, polar bears integrate the status of lower trophic levels. For example, changes in biodiversity among lower trophic levels are reflected in the health of consumers at the top of the Arctic marine food web (e.g. "bottom-up" effects). Thus, polar bears provide a means to assess the health of a largely invisible and inaccessible marine sub-sea-ice ecosystem. Species that are high on the food web are also most comparable to humans conducting subsistence activities in the Arctic. Futhermore, they are the most sensitive to bioaccumulative and biomagnifying stressors. Humans are also apex predators in the Arctic marine food web, thus polar bears can serve as proxies for assessing impacts of changes in the arctic ecosystem upon subsistence users. Because human arctic residents and polar bears both feed at the apex of the ecosystem (consuming many of the same species), polar bears can serve as indicators of human health in the Arctic, regarding access to and "quality" of subsistence foods (e.g. low in contamiants, free of disease.

A major challenge of using apex predators as sentinels is that such species are often rare and difficult to study. Efforts to assess polar bear health, for example, are quite costly and

logistically challenging. Furthermore, only minimally invasive sampling techniques may be used, limiting the amount of information obtained regarding reproductive status, general condition, health and disease. The arctic fox has a similar feeding ecology as the polar bear and can therefore be used as a "surrogate sentinel" due to its abundance, and because collecting tissues does not impair populations, especially when coordinated with ongoing trapping and nuisance program efforts. In additon, they are well known to thrive around human habitation thus contact with this species and their associated pathogens, is very likely. This makes them particularly useful as a sentinel for changes in disease ecology because they share some pathogens with polar bears and humans [e.g. rabies (with both humans and polar bears), *Echinococcus* (with humans only), morbillivirus (with polar bears only)].

While there are a number of human health concerns related to climate change, this thesis focuses on the use of sentinels to indicate changes in food security and patterns of infectious disease exposure in the Arctic (Figure 1). Figure 2 illustrates the complex interrelatedness of these factors. Change in patterns of infectious disease and in food security impact the health of both humans and apex predators. For example, hazards associated with hunting on sea-ice effect food security directly (e.g. via lowered hunting success, risk of injury) and indirectly as change in sea ice dynamics impact the availability of specific prey species. Increases in contaminant levels in subsistence foods and/or shift in subsistence/prey species, which may occur with warming in the Arctic might result in adverse health outcomes in apex predators. Change in the ecology of

zoonotic disease impacts both the health of humans and of wildlife directly, via morbidity and mortality and indirectly by influencing the availiability of subsistence/prey species. Change in ecology of non-zoonotic disease impacts the health of some apex predators directly and also affects food security of apex predators and humans both.

Using Biomarkers to Evaluate Ecosystem Health

The development of diagnostic tools and epidemiology is needed to establish a tool set for evaluating ecosystem health. In many countries, human health data are archived in publicly funded centralized databases and can be retrieved for epidemiologic studies. In contrast, births, deaths, and health status of free ranging animals are largely unobserved and therefore, unrecorded. If recorded, data quality varies and is generally inaccessible. Few animals are found and thoroughly necropsied to ascertain cause of death and/or assess variations in these events over time and space (trends assessment). The process of disease is a continuum from the onset of exposure to the stressor (s) through precursor, compensatory states (e.g. impairment), and clinical illness (or dysfunction), which may result in irreversible impacts or death (Fox, 2001). Change in physiological resilience or tolerance can be subtle and elusive. Often, there is a "tipping point" (defense mechanism is depleted or no longer effective) at which a major insult results from a stressor that is typically not harmful alone. For example, some epizootics occur only in the presence of other stressors (e. g immunosuppression which may result from one or multiple stressors). This "tipping point" perspective is not well appreciated or evaluated. However, morbidity and mortality lead to population level consequences (i.e. alterations in

abundance and distribution, age and sex ratios, genetic diversity) and potential extinctions of local populations are then noted. Monitoring for the impairment of physiologic (molecular, biochemical, cellular) and behavioral responses can provide early warning before impacts occur at the population level and thus allow for effective management intervention. Biomarkers, which can assess the integrated impact of stressors, are of great utility for such monitoring, but underutilized.

Biomarkers have been traditionally defined as cellular, biochemical or molecular alterations that are measurable in biological media such as tissues, cells, or fluids (Hulka et al., 1990). This definition was later expanded to include biological characteristics that can be objectively measured and evaluated as indicators of normal biological or pathogenic processes (Naylor, 2003). With the growth and advancement of molecular biology and laboratory technology, the use of technically advanced biomarkers has greatly expanded and now moved well beyond human medicine.

The identification and quantification of individual stressors (e.g. chemicals, pathogens, etc.) and associated risk assessment is time consuming and costly. Furthermore, evaluating individual stressors cannot indicate the synergistic impact of combined stressors. Additionally, some biomarkers can reflect the entire spectrum of disease, from early manifestations to terminal stages. Biomarker data can be combined with demographic measures (population size, survival and recruitment rates, age structure, sex ratios) to develop predictive modeling capacity, identify at risk cohorts, and to facilitate

observation of the cumulative effects of multiple stressors (Fox, 2001). Today, the great utility of biomarkers as tools for ecological health assessment is well recognized. With the "One Health" initiative, the use of such tools has grown vast.

Thesis Objectives

In the following studies, we employ a "One Health" approach for establishing capacity to monitor changes in ecological health on the Alaska North Slope anticipated with climate change. Such questions require the collaborative efforts of veterinarians, chemists, microbiologists, health care providers, as well as land and organism based managers (resource management). Thus, diverse techniques, tools and analytical methodologies were employed, which reflects need for transdisciplinary efforts to address questions regarding ecosystem health. Here we: establish cohort specific baseline data as a tool for sentinel population health assessment using hematological indices; use these indices as a biomarker of exposure to select indicators of infectious agents; examine prevalence patterns and risk factors to these same indicators using serology; and employ molecular epidemiologic techniques to investigate strains of canine distemper virus (CDV) and Taeniid parasites in sentinel arctic canids.

Chapter one develops capacity for modeling trends in polar bear health by providing a benchmark point for a biomarker (hematological indices). Assessing specific biomarkers in polar bears can provide otherwise unavailable insights for resource managers. Long term trends of this biomarker can be used to assess the health of polar bears, as well as

whether there may be cause for concern for the health outcomes of humans. For humans, and domestic and laboratory animals, this biomarker is a well-characterized tool used regularly by health professionals and researchers. For polar bears, we established these measures for specific age and reproductive cohorts before venturing into a response measure. Chapter two examines associations of hematological parameters (biomarker from Chapter 1) in polar bears with titers to two "indicator" infectious agents present in North Slope predators. In doing so, we relate a response metric to exposure instead of simply reporting seroprevalence, thus advancing these efforts to provide more relevance to potential impacts.

In chapter three, the arctic fox was examined to investigate the strain of CDV circulating on the North Slope of Alaska, because we did not have access to infected tissue (only blood) in polar bears or other potential carnivore hosts in the area. In chapter four we confirm the identity of and provide benchmark prevalence data for Taneiid spp. currently on the Alaska North Slope. Taeniid tapeworms (Eucestoda: Cyclophyllidea: Taeniidae) are parasites of mammals, with carnivores as definitive and mostly herbivores as intermediate hosts (Lavikainen et al., 2008). Because of their medical and veterinary significance, with respect to morbidity and mortality in humans and production losses to domestic food animals worldwide, taeniids have been the focus of intensive epidemiological, ecological and taxonomic studies (Hoberg, 2002). This research has utility for medical epidemiologists because echinococcosis is of particular public health concern in the Arctic. We also demonstrate the advantages of using canid definitive hosts

as sentinels for risk of human infection with these significant parasite zoonoses.

These studies establish a "tool set" for assessing change in ecosystem health anticipated with climate change on the Alaska North Slope with a focus on apex ursid and apex canid predators. Such devices have utility in informing both public and wildlife population health. The evaluation of biomarker responses in sentinel species can provide valuable insights into alteration in ecosystem health if the proper multidisciplinary context is maintained to assure the best analysis and interpretation of these data. These efforts require logistical and intellectual inputs from the field (community) to the laboratory. To this end, this thesis strives to coalesce the relevant biological variables in an ecological context in the analysis and interpretation of these data. Through these studies, we hope to illustrate the value of using the "One Health" approach to answer questions regarding the impact of climate change on ecosystem health in the Arctic.

References

Aguirre AA. 2009. Wild canids as sentinels of ecological health: A conservation medicine perspective. Vectors and Parasites 2(1):S7 doi:10.1186/1756-3305-2-S1-S7.

AMAP. 2003. AMAP Assessment 2002: The Influence of global change on contaminant pathways to, within, and from the Arctic, Oslo, Norway: Arctic Monitoring and Assessment Programme (AMAP).

Amstrup SC. 2003. Polar bear (*Ursus maritimus*) In: Wild Mammals of North America-Biology, Management, and Conservation, Feldhamer GA Thompson BC, Chapman JA (editors), Baltimore, MD: John Hopkins University Press, pp 587-610.

Amstrup SC, Marcot BG, Douglas DC. 2008. A Bayesian network modeling approach to forecasting the 21st century worldwide status of polar bears. In: Arctic Sea Ice Decline: Observations, Projections, Mechanisms, and Implications. Geophysical Monograph Series 180, DeWeaver ET, Bitz CM, Tremblay LB (editors), Washington, DC: American Geophysical Union, pp 213-268.

Backer LC, Grindem CB, Corbett WT, Cullins L, Hunter JL. 2001. Pet dogs as sentinels for environmental contamination. Science of the Total Environment 274(1-3):161-169.

Blais JM, Schindler DW, Muir DCG, Sharp M, Donald D, Lafrenière M, Braekevelt E, Strachan WMJ. 2001. Melting Glaciers: A major source of persistent organochlorines to subalpine Bow Lake in Banff National Park, Canada. Ambio 30(7):410-415.

Carson R. 1962. Silent Spring, Boston: Houghton Mifflin.

Comiso JC. 2006. Abrupt decline in the arctic winter sea ice cover. Geophysical Research Letters 33: L18504, doi:10.1029/2006GL027341 [Online August 21, 2006].

Costello A, Abbas M, Allen A, Ball S, Bell S, Bellamy R, Friel S, Groce N, Johnson A, Kett M, Lee M, Levy C, Maslin M, McCoy D, McGuire B, Montgomery H, Napier D, Pagel C, Patel J, de Oliveira JA, Redclift N, Rees H, Rogger D, Scott J, Stephenson J, Twigg J, Wolff J, Patterson C. 2009. Managing the health effects of climate change: Lancet and University College London Institute for Global Health Commission. Lancet 373(9676):1693-733.

Evander M, Ahlm C. 2009. Milder winters in northern Scandinavia may contribute to larger outbreaks of haemorrhagic fever virus. Global Health Action doi:10.3402/gha.v2i0.2020 [Online November 11, 2009].

Fox G. 2001. Wildlife as sentinels of human health effects in the Great Lakes-St. Lawrence Basin. Environmental Health Perspectives 109(6):853-861.

Gosselin M, Levasseur M, Wheeler PA, Horner RA, Booth BC. 1997. New measurements of phytoplankton and ice algal production in the Arctic Ocean. Deep-Sea Research II 44(8):1623-1644.

Hoberg EP. 2002. *Taenia* tapeworms: their biology, evolution and socioeconomic significance. Microbes and Infection 4(8):859–866.

Holland MM, Bitz CM, Tremblay B. 2006. Future abrupt reductions in the summer Arctic sea ice, Geophysical Research Letters 33:L23503; doi:10.1029/2006GL028024 [Online December 6, 2006].

Hulka BS. 1990. Overview of biological markers. In: Biological Markers in Epidemiology, Hulka BS, Griffith JD, Wilcosky TC (editors), New York, NY: Oxford University Press, pp 3–15.

IPCC. 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Solomon S, Qin D, Manning M, Chen Z, M. Marquis, Averyt KB, Tignor M, Miller HL (editors), Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Kutz SJ, Hoberg EP, Polley L, Jenkins EJ. 2005. Global warming is changing the dynamics of Arctic host-parasite systems. Proceedings of the Royal Society B: Biological Sciences 272(1581):2571-2576.

Laaksonen S, Pusenius J, Kumpula J, Venäläinen A, Kortet R, Oksanen A, Hoberg E. 2010. Climate change promotes the emergence of serious disease outbreaks of filarioid nematodes. EcoHealth 7(1):7-13.

Lavikainen A, Haukisalmi V, Lehtinen MJ, Henttonen H, Oksanen A, Meri S. 2008. A phylogeny of members of the family Taeniidae based on the mitochondrial cox1 and nad1 gene data. Parasitology 135(12):1457-1467.

Lohmann R, Breivik K, Dachs J, Muir D. 2007. Global Fate of POPs: Current and future research directions. Environmental Pollution 150(1):150–165.

McKinney MA, Peacock E, Letcher RJ. 2009. Sea ice associated diet change increases the levels of chlorinated and brominated contaminants in polar bears. Environmental Science &Technology 43(12):4334-4339.

Michel C, Nielsen TC, Nozais C, Gosselin M. 2002. Significance of sedimentation and grazing by ice micro- and meiofauna for carbon cycling in annual sea ice (northern Baffin Bay). Aquatic Microbial Ecology 30(1):57-68.

Naylor S. 2003. Biomarkers: current perspectives and future prospects. Expert Review of Molecular Diagnostics 3(5):525–529.

Niemi G, Wardrop D, Brooks R, Anderson S, Brady V, Pearl H, Rakocinski C, Brouwer M, Levinson B, McDonald M. 2004. Rationale for a new generation of indicators for coastal waters. Environmental Health Perspectives 112(9):979-986.

Parkinson AJ, Berner J. 2009. Climate change and impacts on human health in the Arctic: an international workshop on emerging threats and the response of Arctic communities to climate change. International Journal of Circumpolar Health 68(1):84-91.

Parkinson CL, Cavalieri DJ. 2008. Arctic sea ice variability and trends, 1979–2006 Journal of Geophysocal Research. Oceans 113, C07003, doi:10.1029/2007JC004558 [Online July 1, 2008].

Portier CJ, Thigpen Tart K, Carter SR, Dilworth CH, Grambsch AE, Gohlke J, Hess J, Howard SN, Luber G, Lutz JT, Maslak T, Prudent N, Radtke M, Rosenthal JP, Rowles T, Sandifer PA, Scheraga J, Schramm PJ, Strickman D, Trtanj JM, Whung P-Y. 2010. A Human Health Perspective On Climate Change: A Report Outlining the Research Needs on the Human Health Effects of Climate Change. Research Triangle Park, NC: Environmental Health Perspectives/National Institute of Environmental Health Sciences. doi:10.1289/ehp.1002272 Available: www.niehs.nih.gov/climatereport [accessed September 5, 2010].

Post E, Forchhammer MC, Bret-Harte MS, Callaghan TV, Christensen TR, Elberling B, Fox AD, Gilg O, Hik DS, Høye TT, Ims RA, Jeppesen E, Klein DR, Madsen J, McGuire AD,Rysgaard S, Schindler DE, Stirling I, Tamstorf MP, Tyler NJ, van der Wal R, Welker J, Wookey PA, Schmidt NM, Aastrup P. 2009. Ecological dynamics across the Arctic associated with recent climate change. Science 325(5946):1355-1358.

Rabinowitz P, Scotch M, Conti L. 2009. Human and animal sentinels for shared health risks. Veterinaria Italiana 45(1):23-24.

Rapport DJ, Costanza R, McMichael AJ. 1998. Assessing ecosystem health. Trends in Ecology and Evolution 13(10):397-402.

Siegel DA, Franz BA. 2010. Oceanography: Century of phytoplankton change. Nature 466(7306):569- 571.

Stirling I, Lunn NJ. 1997. Environmental fluctuations in arctic marine ecosystems as reflected by variability in reproduction of polar bears and ringed seals. In:Woodin SJ, Marquiss M (editors), Ecology of Arctic Environments. Special Publication (13) of the British Ecological Society, Blackwell Science Ltd., Oxford, England, pp. 167-181.

Suk WA, Avakian MD, Carpenter D, Groopman JD, Scammell M, Wild CP. 2004. Human exposure monitoring and evaluation in the Arctic: The importance of understanding exposures to the development of public health policy. Environmental Health Perspectives 112(2):113–120.

Tittensor DP, Mora C, Jetz W, Lotze HK, Ricard D, Berghe EV, Worm B. 2010. Global patterns and predictors of marine biodiversity across taxa. Nature 466(7310):1098-1101.

U.S. Fish and Wildlife Service. 2008. Endangered and threatened wildlife and plants; determination of threatened status for the polar bear (*Ursus maritimus*) throughout its range. Federal Register 73(95):28212–28303.

Verbrugge LA, Middaugh JP. 2004. Use of traditional foods in a healthy diet in Alaska: Risks in perspective. 2nd Ed., Vol.1: Polychlorinated Biphenyls (PCBs) and related compounds. State of Alaska Epidemiology Bulletin, October 25, 2004.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM. 1997. Human domination of earth's ecosystems. Science 277(5325):494–499.

Ytrehus B, Bretten T, Bergsjø B, Isaksen K. 2008. Fatal pneumonia epizootic in musk ox (*Ovibos moschatus*) in a period of extraordinary weather conditions. EcoHealth 5(2):213-23.

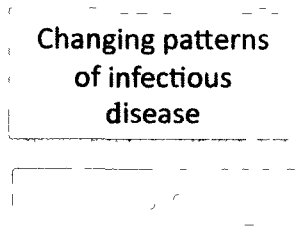

Figure 0.1. Schematic representation of the overlap of human, animal, and ecosystem health.

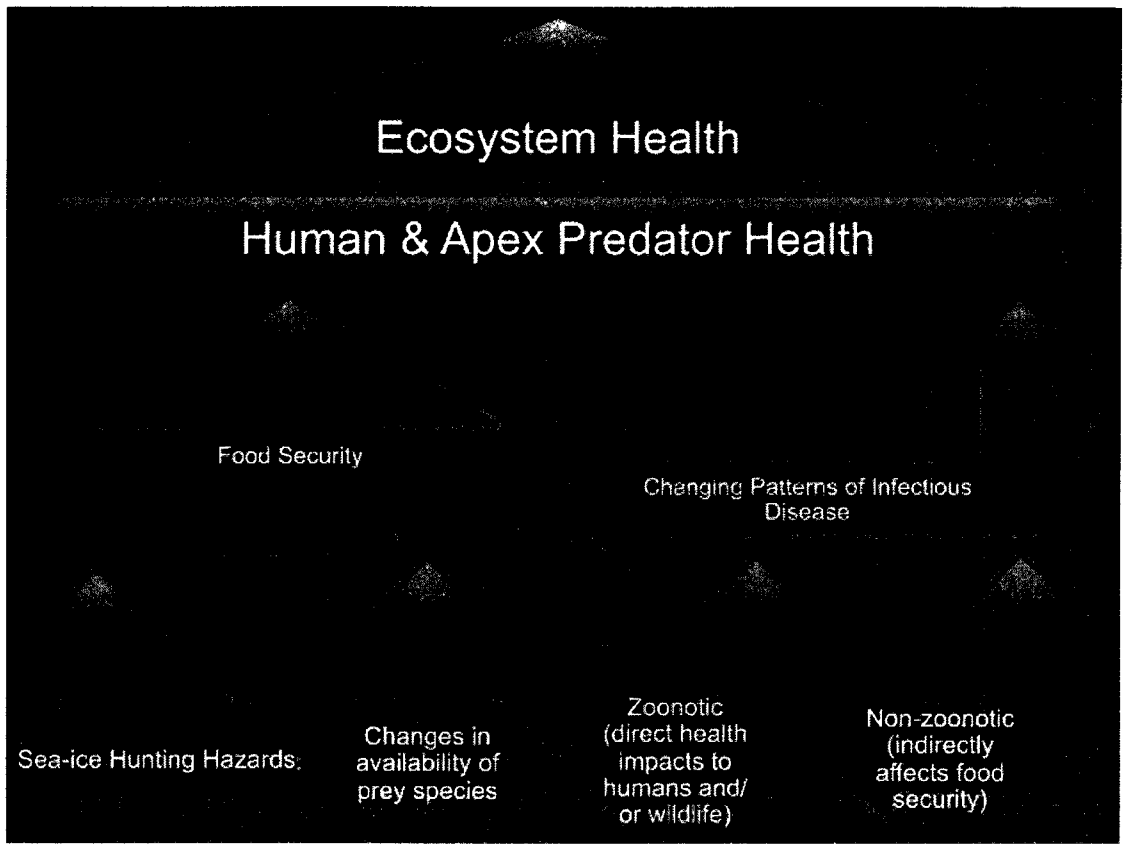

Figure 0.2. Interrelationships among ecosystem, human, and apex predator health.

CHAPTER 1

Hematology of southern Beaufort Sea polar bears (2005-2007): Biomarker for an arctic ecosystem health sentinel[1]

1.1 ABSTRACT

Declines in sea-ice habitats have resulted in declining stature, productivity and survival of polar bears in some regions. With continuing sea-ice declines, negative population effects are projected to expand throughout the polar bear's range. Precise causes of diminished polar bear life history performance are unknown, however climate and sea-ice condition change are expected to negatively affect polar bear (*Ursus maritimus*) health and population dynamics. As apex predators in the Arctic, polar bears integrate the status of lower trophic levels and are therefore sentinels of ecosystem health. Arctic residents feed at the apex of the ecosystem, thus polar bears can serve as indicators of human health in the Arctic. Despite their value as indicators of ecosystem welfare, population-level health data for U.S. polar bears are lacking. We present hematological reference ranges for southern Beaufort Sea polar bears. Hematological parameters in southern Beaufort Sea polar bears varied by age, geographic location and reproductive status. Total leukocytes, lymphocytes, monocytes, eosinophils and serum immunoglobulin G

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were significantly greater in males than females. These measures were greater in non-lactating females ages ≥5 than lactating adult females ages ≥5; suggesting females encumbered by young may be less resilient to new immune system challenges that may accompany ongoing climate change. Hematological values established here provide a necessary baseline for anticipated changes in health as arctic temperatures warm and sea-ice declines accelerate. Data suggest that females with dependent young may be most vulnerable to these changes and should therefore be a targeted cohort for monitoring in this sentinel.

1.2 INTRODUCTION

Observed (Comiso, 2006) and projected (Holland et al., 2006) sea-ice declines led to the conclusion the future welfare of polar bears may be diminished (Amstrup et al., 2008) and to their listing as a threatened species (*U.S. Fish and Wildlife Service*, 2008). Polar bears require sea-ice for prey access and other aspects of their life history (Stirling and Lunn, 1997; Amstrup, 2003). Body condition and stature changes have been reported for southern reaches of polar bear range and in the more northerly polar basin (Stirling et al., 1999, Rode et al., 2010). Observed declines in sea-ice availability associated with reduced body condition have been linked to declines in reproduction, survival, and population size of polar bears (Stirling et al., 1999; Obbard et al., 2006; Stirling and Parkinson, 2006; Regehr et al., 2009). Continued habitat reduction is likely to increase nutritional stress among polar bears (Derocher et al., 2004; Amstrup et al., 2008, 2009). Climate warming, the concomitant alterations in environmental pathways and anticipated changes in nutritional status are expected to increase exposure and susceptibility of polar bears to an increased variety of disease agents (MacDonald, 2003; Derocher et al., 2004; Burek et al., 2008).

Arctic climate historically limited biodiversity of infectious agents and limited life cycles of those present (Kutz et al., 2005). Hence, polar bears are likely immunologically naïve to a significant portion of new disease agents they will encounter with climate change driven entry via abiotic and anthropogenically driven abiotic routes. This raises concern that increasing temperatures, human activities, humidity and frequency of extreme climatic events will cause direct physiological impacts and result in range changes of

disease vectors and intermediate hosts. For example, ballast water from large vessels is a well-known means for introduction of pathogens to new environments (van Riper, 1991). Anticipated expansions in vessel traffic may introduce new organisms concurrent with altered migration patterns and prey availability (MacDonald, 2003). With range shift and diminishing sea-ice, polar bears may access alternate and potentially less suitable prey species such as the harbor seal (*Phoca vitulina*) and their concomitant pathogens (Derocher et al., 2004). In addition, polar bears may begin to eat more of the intestines and internal organs of seals as they become nutritionally stressed, thereby exposing them to an increased burden and variety of pathogens (Derocher et al., 2004). Increased inter- and intra-species interactions, as higher densities of polar bears spend more time aggregated onshore will lead not only increased pathogen transmission opportunities, but also greater human-wildlife conflict. For example, presence of landfills and hunter-killed whale carcasses has concentrated polar bears, arctic fox, and possibly, domestic canines in close proximity to humans (Bentzen et al., 2008).

The polar bear is a circumpolar top predator of the arctic marine ecosystem (Amstrup, 2003) and thus presents an ideal sentinel for arctic marine ecosystem health (Bossart, 2006). Polar bears integrate lower trophic levels and thus reflect ecosystem health (Grosell and Walsh, 2006). They provide an opportunity to assess the health of a largely invisible and inaccessible marine sub-sea-ice ecosystem. Humans are also apex predators in the arctic food chain, thus polar bears can serve as proxies for assessing impacts of changes in the arctic ecosystem on subsistence users. The dearth of information concerning impacts of

ongoing changes in the Arctic and the difficulty of gathering information in these remote ice-covered areas underscores the need to identify and monitor sentinel species. Polar bears can provide otherwise unavailable insights for resource managers (Bonde et al., 2004). Leukocytes are an essential part of the immune system that protect the organism from infectious agents. Hematology represents valuable tools for evaluating general health status and response to infectious agents of free ranging wildlife. These measures include increases and decreases in general and/or specific cell type populations as concentrations (cells/μl) or as proportions (%) of populations. As a biomarker for monitoring the health of sentinel species, they in turn serve as indicators of ecosystem health. Although such physiological indices can detect changes in animal health before they are apparent at the population level, knowledge of immune system status of polar bear populations is inchoate. Descriptions of hematological indices, the blood values that indicate immune system status, are limited and sample sizes are small or absent and do not provide adequate reference for future change. Because polar bears reflect the health of their ecosystem, knowledge of "normal hematological values" will be an essential part of monitoring and response to projected changes (Aguirre et al., 2002).

Here we provide a hematological baseline for the southern Beaufort Sea subpopulation of polar bears. This baseline provides a yardstick against which hematology studies of the future can be measured and will assist in the use of the polar bear as the "canary in the [Arctic] coal mine" (Tabor and Aguirre, 2004; Bonde et al., 2004). The following companion paper establishes seroprevalence, patterns for exposure risk and associations

with hematological biomarkers for two indicator pathogens (morbillivirus and *Toxoplasma gondii*) chosen based on previous documentation of antibodies to these agents in this species as well as their potential to be altered by climate change in the Arctic ecosystem.

1.3 METHODS

1.3.1 Animal Capture

We captured polar bears by injection of Telazol® in darts fired from helicopters (Stirling et al., 1989). Studies occurred in the southern Beaufort Sea from late March through early May 2005, 2006 and 2007. Study area encompassed the region between Barrow (157°00' W) and Demarcation Point (141°00' W) and up to approximately 80 km off shore (Figure 1.1). Capture missions were launched from logistics bases at Barrow, Prudhoe Bay, and Kaktovik with the exception of 2006 (Kaktovik). Daily routine of launching and returning assured some clustering of captures near each of these locations. Capture procedures were approved by the U.S. Geological Survey, Alaska Science Center, Institutional Animal Care and Use Committee.

Ages of captured polar bears were known for animals first captured with their mothers as dependent young. Ages of independent animals were determined by counting cementum annuli of an extracted vestigial premolar (Calvert and Ramsay, 1998). Each bear was marked with a unique tattoo and ear tag number upon first capture assuring individual

identification upon recapture. We classified age classes representing distinct life history stages (Amstrup, 2003): 1-2 years of age (dependents captured with mother); 3-4 years (weaned, pre-breeding age); 5-7 years (age of sexual maturation); and ≥ 8 years of age (greater likelihood of reproductive success) (Amstrup, 2003). First year animals (cubs) were not included in our study. We compared reproductive cohorts: females ≥ 5 years with dependent young (lactating), solitary females ≥ 5 years (non-lactating), and males ≥ 5 years. Recognizing potential differences in exposure to human influences (discarded foods, stress from human encounters) we compared hematological parameters among the three logistics bases.

1.3.2 Blood Collection and Processing

We used evacuated tubes (Vacutainer, BD Biosciences; Franklin Lanes, NJ) for blood collection by venipuncture of the femoral vein or artery. Sera derived from blood without anticoagulant were separated by centrifugation at 1,500 x g for 5 minutes (TRIAC, Clay Adams Co., Parisippany, NJ, USA) and frozen at -20°C. Samples were designated as lipemic if a "fat plug" formed between the clot and serum. Sera were stored at -70°C upon return from the field. We prepared slides for leukocyte differentials, and determined total hematocrit, plasma protein and leukocyte counts using blood collected into EDTA treated tubes. These measures were determined on the day of capture to avoid time dependent post-sampling changes.

1.3.3 Hematology

We determined leukocyte type counts as percentages to provide a differential profile for each polar bear and calculated absolute counts. The differential profile reveals the relative abundance of each cell type, while the absolute concentration expresses how many cells of each type are present per volume (μl) of blood. Differential blood cell counts were performed using blood smears stained with Wright's-Giemsa by identifying and classifying 100 leukocytes into the specific morphologic types (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). Three independent readers conducted differential counts and statistical bias was assessed by plotting values obtained by readers against one another using the most experienced reader's value as the independent variable. Counts were statistically unbiased among readers and therefore, count values obtained for all readers were averaged for each individual animal. Total leukocyte counts were determined microscopically using the Unopette® system (BD Biosciences; Franklin Lanes, NJ, USA) and hemocytometer according to manufacturer's instructions. Absolute counts for specific cell types were calculated as the product of mean percentage as determined in the differential count (e.g. % neutrophils) and total leukocyte count for each individual. We determined hematocrit for EDTA treated whole blood using a high-speed micro-centrifuge for 3 minutes at 12,600 x g (TRIAC; Clay Adams Co., Parisippany, NJ, USA) and measuring the resulting packed cells as a percent of blood volume using Critocaps® chart-type microhematocrit tube reader (McCormick Scientific; St. Louis, MO, USA) taken in duplicate for each animal and averaged. We compared our data to hematological reference ranges reported in the International Species

Information System (ISIS, www.isis.org; 200 samples collected from 90 individual polar bears) and the CRC Handbook of Marine Mammal Medicine where Cattet and Caulkett (2001) contributed hematological ranges (no means) for between 15 and 181 free-ranging Churchill, Manitoba (Canada) polar bears. Hematocrit values were compared to those for Svalbard (Tryland et al., 2002) and Manitoba (Lee et al., 1977) polar bears; and to previously unpublished hematocrit values measured for Beaufort Sea polar bears between 1982 and 1988.

Immunoglobulin G (IgG) from polar bears was isolated by adsorption of serum IgG on a Protein G Sepharose 4 Fast Flow column (Sigma-Aldrich) and subsequent desorption using a reducing pH gradient starting with 0.01M PBS, pH 7.4 and then an increasing proportion of 0.1M citric acid, pH 2.7 was added (Bernhoft et al., 2000). Purified IgG was quantified using Pierce BCA protein assay kit and sent to Cocalico Biologicals, Inc.™ (Reamstown, PA, USA) for generation of goat and rabbit-anti polar bear IgG. Specificity of antiserum generated was assessed via immunoelectrophoresis. Serum IgG concentration was measured using a single radial immunodiffusion assay as described previously (Mancini et al., 1964). A dilution series (1,000 mg/ml, 800 mg/ml, 600 mg/ml, 400 mg/ml, and 200 mg/ml) of polar bear IgG was run on each plate to serve as a standard curve. An aliquot of pooled polar bear serum was also run on each plate to serve as a positive control and each serum sample was run in duplicate on independent plates. The diameters of the precipitation zones were evaluated using a "measuring viewer"

(Behring Institut, Germany), and IgG concentrations (mg/ml) calculated from the standard curve on each corresponding plate.

1.3.4 Statistical Analyses

Summary statistics were calculated and normality assessed using boxplots and the Shapiro-Wilk goodness of fit test (JMP 7; SAS Institute, Cary, NC, USA). We used the Grubb's test to determine the significance of outliers. Suspected outliers with lack of significant deviation were included in the calculation of means, 95% confidence interval, median, standard deviation, and range. Some individuals were excluded on the basis of missing values. Nine bears (<5% of those captured) were recaptured and re-sampled during the study. Correlation analysis demonstrated independence of sample measurements over time. Reference ranges were calculated with and without these replicate measures, the maximum difference in any calculated range was less than 2%. Therefore, reference ranges reported here include all samples. Log transformations were employed where data were not normally distributed. We compared means among age and reproductive cohorts and across years and locations using analysis of variance and post-hoc Tukey-Kramer test for normally distributed variables and the Kruskal-Wallis test for non-normal data. Homogeneity of variance among groups was assessed using O'Brien's, Levene's and the Brown-Forsythe test(s). We used the Welch test to compare groups where variance was unequal. We also used the post-hoc Dunnett-Tukey-Kramer pairwise multiple comparisons test to determine which groups were significantly different (R Development Core Team, 2006). Student's t-tests were used for intersex comparisons

(JMP 7; SAS Institute, Cary, NC, USA). Interaction between variables (age, sex, location and year) and each hematologic parameter (e.g. hematocrit) were explored via regression utilizing a backwards stepwise approach and Akaike's Information Criteria for model selection where age is a continuous variable, sex is male or female, year is 2005, 2006, 2007 and location is Barrow, Prudhoe or Kaktovik (R Development Core Team, 2006). Linear relationships between age (continuous variable) and all parameters were assessed using simple bivariate plots.

Many statistically significant differences among sex and age classes were small, thus we emphasize that statistical significance among reference values may not imply biological significance. This is partly explained by the fact that the null hypothesis (samples being compared are identical) is probably seldom true (Heath, 1995), and when conducting a large number of statistical tests, some will appear significant by chance alone. Therefore, to avoid over-interpretation, we quantified differences among reference values as a percentage. We calculated percentage differences subtracting the first parameter from the second, dividing the difference by the first parameter and multiplying by 100. To be consistent with regard to possible direction of differences, parameters were designated as "first" or "second" based upon sex and age status. There are no established rules dictating how large a difference is required to achieve biological importance. We selected biological significance of differences smaller than 20% as questionable and that differences $\geq$20% are noteworthy.

1.4 RESULTS

We analyzed hematological data from 98 female and 91 male polar bears captured in the southern Beaufort Sea of Alaska (2005, 2006, 2007). We observed no overt clinical signs of disease upon gross examination. Reference values for all polar bears across sample years and cohorts are presented (Table 1.1a). Mean values for total leukocytes, neutrophils, lymphocytes, monocytes, leukocyte differential counts, and hematocrit measured in the springs of 2005 (n=63), 2006 (n=68), and 2007 (n=59) are within one standard deviation of values reported for captive polar bears (ISIS Database) (Table 1.1b). Eosinophil and basophil counts were less than those reported by ISIS and free-ranging Churchill, Manitoba polar bears (Catett and Caulkett, 2001) (Table 1.1a-c). IgG measured for southern Beaufort Sea polar bears fell within one standard deviation of that reported for Svalbard, Norway polar bears (Bernhoft et al., 2000).

Several parameters varied significantly between sexes. Total leukocyte, neutrophil, monocyte, and eosinophil counts and IgG were significantly greater in males than females (all age classes and reproductive cohorts are combined, Table 1.2 a-c). We also noted significant differences between lactating and non-lactating females (Table 1.3). Total leukocytes; lymphocytes, monocytes, eosinophils (absolute count and by % of 100 cells); and IgG were significantly greater in non-lactating females ages ≥5 than lactating adult females ages ≥5. When males aged ≥5 were compared to each female reproductive cohort, total leukocytes, absolute counts for lymphocytes; neutrophils, monocytes, eosinophils and IgG were significantly greater than that of lactating females. Percent

lymphocytes for males aged ≥5, however, were lower than that of lactating females. No biologically significant differences were evident between males ages ≥5 and non-lactating females ages ≥5. We also noted differences among age cohorts (Tables 1.4a-d and Table 1.4e). Neutrophil counts were significantly greater in bears ≥ 8 than ages 5-7. Lymphocyte counts were significantly greater in polar bears aged 1-2, 3-4 and 5-7 than bears aged ≥ 8 years. Percent neutrophils were greater in individuals ≥ 8 than age cohorts 3-4 and 5-7. Bears aged 3-4 displayed significantly greater percentage eosinophils than ages 1-2. We observed significantly greater percent lymphocytes in polar bears aged 1-2, 3-4 and 5-7 than ≥ 8 years. Total IgG was significantly greater in ages ≥ 8 than all younger bears. Regression analyses revealed that log, neutrophils, and IgG increased with age while log (lymphocytes) and lymphocytes decreased with age. R^2 values were less than 20%. Hematology parameters did not differ significantly across years. Mean monocyte counts varied among locations (Welch test p >0.0279). Dunnett-Tukey-Kramer pairwise multiple comparison test (adjusted for unequal variances and sample sizes) revealed that only Kaktovik and Prudhoe samples were significantly different (Figure 1.2).

1.5 DISCUSSION

We report hematology values for free ranging polar bears with sample size sufficient to establish "normal" ranges for specific sex, age, and reproductive cohorts. Establishing normal baseline ranges for these parameters is necessary to monitor trends in polar bear health and for assessing impacts of toxicants, infectious agents, nutrients, etc. The ability

to better understand and monitor polar bear health will allow researchers and managers to use the polar bears' sentinel status as a window into arctic marine ecosystem health.

Leukocyte data have been available for captive polar bears (ISIS) but sample sizes were potentially compromised by repeated measures and variation in animal care regimens among captive institutions. The utility of these data from captive bears for assessing free ranging bears may be limited. Insufficient data have previously been available from wild bears to establish useful baselines or to compare with values from captive animals. For example, studies in Manitoba, Canada and Svalbard, Norway (N=33, Lee et al., 1977; N=31 Tryland et al., 2002) reported biochemical values including hematocrit for wild polar bears. Sample sizes, however, were limited and leukocyte profiles were not available. Leukocyte data available from free-ranging Manitoba polar bears (Cattet and Caulkett, 2001) reported sample sizes ranging from 18 to 151 without details (e.g. mean values or measurements of variation) necessary for making comparisons among cohorts or geographic regions. This lack of detail in previously published data limits the ability of using polar bears as sentinel species for arctic ecosystem health.

Most equipment used (e.g. clinical analyzers) for assessing blood cell types is calibrated for domestic species presenting technical challenges in measuring blood cell types for wildlife. The majority of species have unique blood cell morphology and, therefore, using a clinical analyzer that is, for example, calibrated for canines, to determine polar bear leukocyte profiles, could result in erroneous measurements. Thus we used traditional

microscopy wherein cell types were determined by multiple observers familiar with leukocyte morphology for a variety of species. Previously published values lack details on methodology, sample size and descriptive statistics; our dataset provides means, SD, and 95% CI; parameters necessary for statistical comparisons among specific cohorts (age, sex, reproductive status) among polar bear populations and over time.

Many factors including nutrition, genetics and stress influence hematology values. Despite differences between captive and free ranging polar bears (e.g. activity level, nutrition, environment) several hematological parameters were similar. Differences between our data and those observed for ISIS and Churchill, Manitoba (Cattet and Caulkett, 2001) polar bears in eosinophils and basophils may be due to differences in methodologies in animal capture, sample collection and measurement, environment and exposure and/or stochasticity, or a combination thereof. The absence of details regarding previously published values complicates assessment of variation.

Because hematological analyses for captive bears are primarily conducted using automated analyzers, differences in parameter values may reflect variation in equipment, reagents, and calibration across institutions and time. A description of methods used to count cell types for Manitoba bears (Cattet and Caulkett, 2001) was unavailable, but seasonality of sample collection, variation in environmental conditions and antigen exposure and other unknown factors could contribute to differences observed between

sample populations. Cell types for which differences were observed are present in smaller numbers and therefore stochastic effects could play a role.

Variation we report may reflect ecological differences among sex and age classes of polar bears. We sampled polar bears in spring when females with cubs emerge from dens, when non-lactating females come into estrous, and when naïve ringed seal (*Phoca hispida*) pups are most vulnerable to predation. At this time, female polar bears are focused on taking advantage of foraging opportunities. Den emergent females must restore body reserves lost during hibernation, gestation and lactation. Sexually mature males, however, often forego favorable feeding opportunities to focus on finding mates. Because males may be able to breed at 3 years of age (Rosing-Asvid et al., 2002; Cronin et al., 2009), the physiological effects of voluntary spring fasting may show up early in life. Hence, in addition to possible differences between the sexes, seasonal differences would be expected. Because our 2005-2007 samples were collected in spring only, we could not address seasonal differences. Comparing hematocrit values from our recent samples to values from samples collected in late winter, spring, and fall of 1982-1988 did confirm hematocrit values measured in the 1980s were higher in fall than in late winter or spring. Hematocrit provides an evaluation of red blood cell status and can indicate anemia (numerous causes, e.g. nutrition), blood loss, or dehydration. This corresponds with the fact that Beaufort Sea polar bears are in their best condition in fall and poorest in late winter and early spring (Durner and Amstrup, 1996). Mean hematocrits for Beaufort Sea bears in the current study, (limited to spring captures) were similar to those

determined for spring captured bears in 1982-1988 (Tables1.1c). The recent hematocrit measurements were also similar to those determined for free ranging fall captured Manitoba polar bears. The similarity between many of our values from spring captured polar bears in the Beaufort Sea and those captured during an extended fast when Manitoba bears are trapped on land during a protracted period of sea-ice absence (Lee et al., 1977; Table 1.1b), provides evidence that late-winter and early spring is a period of physiological limitation for polar bears in the Beaufort Sea.

Sex steroids may play a role in the sexual dimorphism of hematologic (immune) patterns. For example, human females typically have decreased cell-mediated immunity responses than males (Schuurs and Verheul, 1990). We detected numerous parameter differences between sexes in our study. However when data were subdivided by sex as well as reproductive status, it appeared that the majority of sex differences were attributed to lactation and/or care of dependent young. With the exceptions of neutrophils and lymphocytes measured by percentage, the same differences demonstrated between males aged ≥5 and lactating females aged ≥5 were present between non-lactating females and lactating females aged ≥5. Lymphocytes (%) were greater in non-lactating females than lactating females, but lower in males aged ≥5 than lactating females (Table 1.3). Decreased numbers can indicate an immunosuppressive condition. Neutrophils (most numerous circulating leukocyte) may increase in response to bacterial infection, inflammatory disease, and stress, whereas decreased neutrophil levels may result from severe and/or chronic infection.

Lactation and dependent young impose both direct and indirect physiological burdens on female polar bears, which appear to affect hematologic measures and maintenance of homeostasis. Such burdens may include: nutritional stress due to lactation, sharing of food resources, and hypothalamic-pituitary axis mediated release of corticosteroids due to stress from care and protection of young. Thus, this cohort may be the most sensitive and vulnerable to stressors, making it the most useful and critical monitor for a stressed ecosystem (e.g. climate change, loss of sea-ice). Several hormones and factors associated with the need for fetal tolerance occur during pregnancy and parturition impacting immune function. During pregnancy cell-mediated immune responses are decreased, likely due to elevation in estradiol levels (Prieto and Rosenstein, 2006). At parturition, progesterone and estradiol levels decrease (Kaker et al., 1984) and plasma cortisol, which can impair polymorphonuclear neutrophils, increases by four-fold (Dosogne et al., 1999).

Neutrophils tend to decrease with age while lymphocytes increase (Thrall et al., 2004). Our data reflect this trend as the number of neutrophils was significantly greater in polar bears aged ≥ 8 than ages 5-7 and lymphocytes were significantly greater in bears of each younger age cohort than bears aged ≥ 8 years. For eosinophils, there was a percentage difference between the youngest two age cohorts of over 100%. Eosinophils may increase in response to allergic disorders, inflammation of the skin, and parasitic infection. Age related change in this cell type has not previously been recognized in mammals. Hence, larger sample sizes will be required to establish whether this finding is noteworthy or spurious.

Circulating total immunoglobulin class G (IgG) comprises all of specific antibodies that have been produced in response to exposure to infectious agents and toxins throughout an organism's lifetime. Hence, increasing levels of IgG with age is an expected result since the likelihood of antigenic exposure should increase over time and many immunological responses generate antibodies with long half-lives. Our analyses suggest the observed sex difference in IgG, like other hematological values, may be driven in part by lactation and/or care of dependent young, which emphasizes the need to assess this specific cohort. Bernhoft et al. (2000) also determined total IgG to be greater in males than female polar bears. They compared adult females to females with cubs of the year in order to determine whether fat loss due to denning may contribute to intersex difference, but found no change.

Monocyte counts were highest in polar bears sampled near Kaktovik however, significance was only achieved for disparity between Kaktovik and Prudhoe. Monocyte levels increase in response to a variety of infections and to inflammatory disorders (Murphy et al., 2005). Differences in monocyte numbers between sampling locations may be attributed to geographical or biological (e.g. antigen) variants of location sites, and/or may be associated with disparity in capture stress between locations. The Kaktovik area includes the presence of subsistence hunter-killed bowhead whale carcasses which draw polar bears close to human settlements and dump sites, thereby facilitating interaction with grizzly bears and conspecifics (Miller et al., 2006; Bentzen et al., 2008). Congregation of polar bears and grizzly bears, arctic and red foxes, and gull species at

carcass may increase risk of intra- and interspecies disease transmission (Miller et al., 2006). Increased monocytes in polar bears captured near Kaktovik may reflect exposure to disease agents due to the occurrence of such anthropogenic food sources. Such supplemental feeding also occurs, although at a less predictable level, near Barrow. This might explain the lack of differences in monocyte counts between Barrow and Kaktovik bears.

Capture protocols and sequence of biological sample collection were consistent across logistic bases. Because Kaktovik was always sampled late in the spring, there may have been a temporal effect that caused variation in hematologic responses. Monocytosis is associated with chronic inflammation, bacteremias and sometimes with corticosteroid responses (Thrall et al., 2004). Stress or corticosteroid-induced leukograms are marked by mature neutrophilia, lymphopenia, and sometimes eosinopenia. Monocyte numbers are generally variable in stress leukograms; however, monocytosis often occurs in steroid or stress reactions in dogs and horses (Aiello and Mays, 1998). We were unable to adequately assess the possibility that the variation we observed might be attributed to a stress leukogram because a normal leukogram for polar bears has not previously been defined. However, when we examined leukograms by location, lymphopenia and eosinopenia in Kaktovik bears were not apparent.

1.6 CONCLUSION

Increasing anthropogenic stressors faced by polar bears include: climate warming (altering sea-ice dynamics); proximity to growing industrial complexes, communities and domestic species; contaminants (long-range transport) and local sources such as the petroleum industry (MacDonald, 2003). Alteration in climate is likely to expose polar bears to an increased variety of pathogens (Derocher et al., 2004; Kutz et al., 2005). Here we provide a "benchmark" for hematological parameters for southern Beaufort Sea polar bears against which variation over time should be assessed. We propose these measures as part of the "tool box" that could be used to assess polar bear health. The polar bears' strategic status as arctic sentinel means these "tools" provide a measure of the status of the whole arctic marine and coastal ecosystems.

Age related variation in hematologic parameters are consistent with those observed in other mammalian species. Lowered counts of some leukocyte types in female polar bears with dependent young suggest this cohort may be less resilient than other sex and age groups to new challenges that may accompany ongoing ecosystem changes. Lowered resilience of reproducing females may compound the already observed declines in reproductive performance of females related to declining sea-ice availability (Stirling et al., 1999; Regehr et al., 2009). This should be an important component of future polar bear health monitoring, research programs, and conservation monitoring that assesses arctic ecosystem health. The stage is now set to use these hematologic measures as biomarkers that may be used to assess polar bear responses to changing infectious agents,

nutritional status, and toxicant exposure that may result from global warming. Because the polar bear is a legitimate sentinel and icon of the arctic, these biomarkers can provide meaningful measures of the status and health of the arctic and coastal marine ecosystems. The following companion paper compliments this effort by evaluating exposure of polar bears to specific indicator infectious agents.

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1.8 REFERENCES

Aguirre AA, Ostfeld RS, Tabor GM, House CA, Pearl MC (editors). 2002. Conservation Medicine: Ecological Health in Practice, New York, NY: Oxford University Press.

Aiello SE, Mays A. 1998. The Merck Veterinary Manual, Eighth Edition, Whitehouse Station, New Jersey: Merck & Co, Inc.

Amstrup SC. 2003. Polar bear (*Ursus maritimus*). In: Wild Mammals of North America-Biology, Management, and Conservation, Feldhamer GA Thompson BC, Chapman JA (editors), Baltimore, Maryland: John Hopkins University Press, pp 587-610.

Amstrup SC, Marcot BG, Douglas DC. 2008. A Bayesian network modeling approach to forecasting the 21st century worldwide status of polar bears. In: Arctic sea Ice Decline: Observations, Projections, Mechanisms, and Implications. Geophysical Monograph Series 180, DeWeaver ET, Bitz CM, Tremblay LB (editors), Washington, DC: American Geophysical Union, pp 213-268.

Amstrup SC, Caswell H, DeWeaver E, Stirling I, Douglas DC, Marcot BG, Hunter CM. 2009. Rebuttal of "Polar bear population forecasts: a public-policy audit". Interfaces 39(4):353-369.

Bentzen TW, Follmann EH, Amstrup SC, York GS, Wooler MJ, Muir DCG, O'Hara TM. 2008. Dietary biomagnification of organochlorine contaminants in Alaskan polar bears. Canadian Journal of Zoology 86(3):177-191.

Bernhoft A, Skaare JU, Wiig Ø, Derocher AE, Larsen HJS. 2000. Possible immunotoxic effects of organochlorines in polar bears (*Ursus maritimus*) at Svalbard. Toxicology and Environmental Health 59(7):561–574.

Bonde RK, Aguirre AA, Powell J. 2004. Manatees as sentinels of marine ecosystem health: are they the 2000-pound canaries? EcoHealth 1(3):255-262.

Bossart GD. 2006. Marine mammals as sentinel species for oceans and human health. Oceanography 19(2):134-136.

Burek KA, Gulland FMD, O'Hara TM. 2008. Effects of climate change on Arctic marine mammal health. Ecological Applications 18(2) Supplement:S126-134.

Calvert W, Ramsay MA. 1998. Evaluation of age determination of polar bears by counts of cementum growth layer groups. Ursus 10(5):449–453.

Cattet M, Caulkett N. 2001. Ranges of hematology and biochemistry values in free-ranging manatees, polar bears and sea otters. In: CRC Handbook of Marine Mammal Medicine, Second Edition, Dierauf LA, Gulland FMD (editors), Boca Raton, Florida: CRC Press, p 396.

Comiso JC. 2006. Abrupt decline in the arctic winter sea ice cover. Geophysical Research Letters 33: L18504, doi: 10.1029/2006GL027341 [Online August 21, 2006].

Cronin MA, Amstrup SC, Talbot SL, Sage GK, Amstrup KS. 2009. Genetic variation, relatedness, and effective population size of polar bears (*Ursus maritimus*) in the southern Beaufort Sea, Alaska. Journal of Heredity doi:10.1093/jhered/esp061 [Online July 24, 2009].

Derocher AE, Lunn NJ, Stirling I. 2004. Polar bears in a warming climate. Integrative and Comparative Biology 44(2):163-176.

Dosogne H, Burvenich C, Freeman AE, Kehrli ME Jr, Detilleux JC, Sulon J, Beckers JF, Hoeben D. 1999. Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. Veterinary Immunology and Immunopathology 67(1):47-54.

Durner GM, Amstrup SC. 1996. Mass and body-dimension relationships of polar bears in northern Alaska. Wildlife Society Bulletin 24(3):480-484.

Grosell M, Walsh PJ. 2006. Benefits from the sea: sentinel species and animal models of human health. Oceanography 19(2):126-133.

Heath D. 1995. An Introduction To Experimental Design and Statistics For Biology. Boca Raton, FL: CRC Press.

Holland MM, Bitz CM, Tremblay B. 2006. Future abrupt reductions in the summer Arctic sea ice, Geophysical Research Letters 33: L23503; doi:10.1029/2006GL028024 [Online December 6, 2006].

Kaker ML, Murray RD, Dobson H. 1984. Plasma hormone changes in cows during induced or spontaneous calvings and the early post partum period. Veterinary Record 115(15):378-382.

Kutz SJ, Hoberg EP, Polley L, Jenkins EJ. 2005. Global warming is changing the dynamics of Arctic host-parasite systems. Proceedings of the Royal Society B: Biological Sciences 272(1581): 2571-2576.

Lee J, Ronald K, Oritsland NA. 1977. Some blood values of wild polar bears. The Journal of Wildlife Management 41(3):520-526.

MacDonald RW. 2003. How will global climate change affect the risk from long-range transport of persistent organic pollutants? Human and Ecological Risk Assessment 9(3):643-660.

Mancini G, Vaerman JP, Carbonara AO, Heremans JF. 1964. A single-radial-diffusion method for the immunological quantitation of proteins. In: Protides of the Biological Fluids, Peeters H (editor), Amsterdam: Elsevier, pp 370–373.

Miller S, Proffitt K, Schliebe S. 2006. Demography and behavior of polar bears feeding on bowhead whale carcasses at Barter and Cross Islands, Alaska, 2002–2004. Outer Continental Shelf Study, Anchorage, AK: Minerals Management Service, U.S Department of the Interior.

Murphy K, Travers P, Walport M. 2005. Janeway's Immunobiology, 6th edition, New York, NY: Garland Science Publishing.

Obbard ME, Cattet MRL, Moody T, Walton LR, Potter D, Inglis J, Chenier C. 2006. Temporal trends in the body condition of southern Hudson Bay polar bears. Climate Change Research Information Note, No. 3, Sault Ste. Marie, ON, Canada: Ontario Ministry of Natural Resources, 8 pp.

Prieto GA, Rosenstein Y. 2006. Oestradiol potentiates the suppressive function of human CD4 CD25 regulatory T cells by promoting their proliferation. Immunology 118(1):58-65.

R-Development Core Team. 2006. R: A Language and Environment for Statistical Computing, Vienna: R Foundation for Statistical Computing, ISBN 3-900051-07-0. Available: http://www.R-project.org.

Regehr EV, Hunter CM, Caswell H, Amstrup SC, Stirling I. 2009. Survival and breeding of polar bears in the southern Beaufort Sea in relation to sea ice. Journal of Animal Ecology doi: 10.1111/j.1365-2656.2009.01603.x [Online September 14, 2009].

Rode KD, Amstrup SC, Regehr EV. 2010. Reduced body size and cub recruitment in polar bears associated with sea ice decline. Ecological Applications 20(3):768–782.

Rosing-Asvid A, Born EW, Kingsley MCS. 2002. Age at sexual maturity of males and timing of the mating season of polar bears (*Ursus maritimus*) in Greenland. Polar Biology 25(12):878–883.

Schuurs AH, Verheul HA. 1990. Effects of gender and sex steroids on the immune response. Journal of Steroid Biochemistry 35(2):157-172.

Stirling I, Spencer C, Andriashek D. 1989. Immobilization of polar bears (*Ursus maritimus*) with Telazol® in the Canadian Arctic. Journal of Wildlife Diseases 25(2):159-168.

Stirling I, Lunn NJ. 1997. Environmental fluctuations in arctic marine ecosystems as reflected by variability in reproduction of polar bears and ringed seals. In: Ecology of Arctic Environments, Special Publication (13) of the British Ecological Society, Woodin SJ, Marquiss M (editors), Oxford, UK: Blackwell Science.

Stirling I, Lunn NJ, Iacozza J. 1999. Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. Arctic 52(3):294-306.

Stirling I, Parkinson CL. 2006. Possible effects of climate warming on selected populations of polar bears (*Ursus maritimus*) in the Canadian Arctic. Arctic 59(3):261-275.

Tabor GM, Aguirre AA. 2004. Ecosystem health and sentinel species: adding an ecological element to the proverbial "canary in the mineshaft. EcoHealth 1(3):226-228.

Thrall MA (editor), Baker DC, Lassen ED. 2004. Veterinary Hematology & Clinical Chemistry Ames, IA: Blackwell Publishing.

Tryland M, Brun E, Derocher AE, Arnemo JM, Kierulf P, Ølberg RA, Wiig Ø. 2002. Plasma biochemical values from apparently healthy free-ranging polar bears from Svalbard. Journal of Wildlife Diseases 38(3):566-575.

U.S. Fish and Wildlife Service. 2008. Endangered and threatened wildlife and plants; determination of threatened status for the polar bear (*Ursus maritimus*) throughout its range. Federal Register 73(95):28212–28303.

van Riper C III. 1991. The impact of introduced vectors and avian malaria on insular Passeriform bird populations in Hawaii. Bulletin of the Society of Vector Ecology 16(1):59-83.

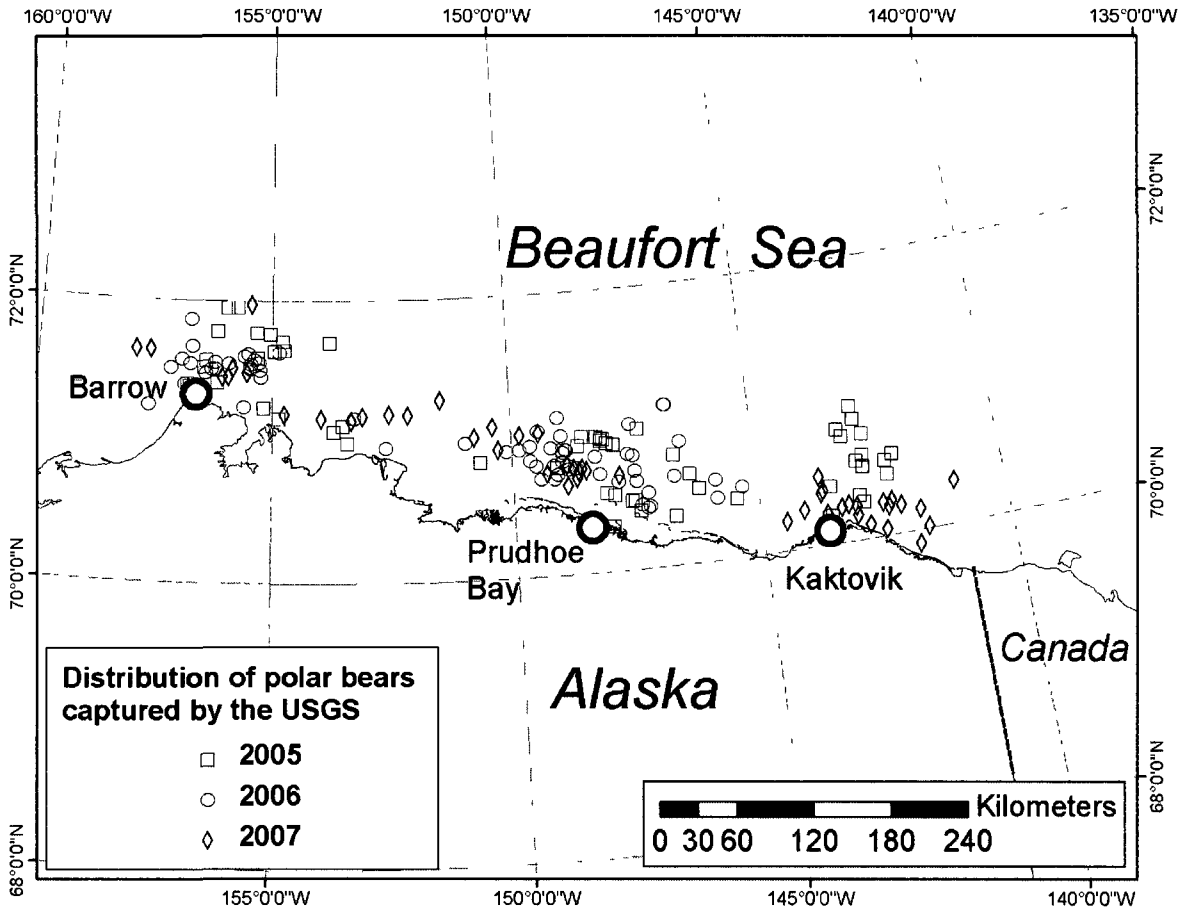

Figure 1.1 Map of northern Alaska and the southern Beaufort Sea, showing polar bear capture areas in spring 2005-2007 from Barrow, Prudhoe Bay and Kaktovik, Alaska. Note: polar bears were not captured out of Kaktovik in 2006.

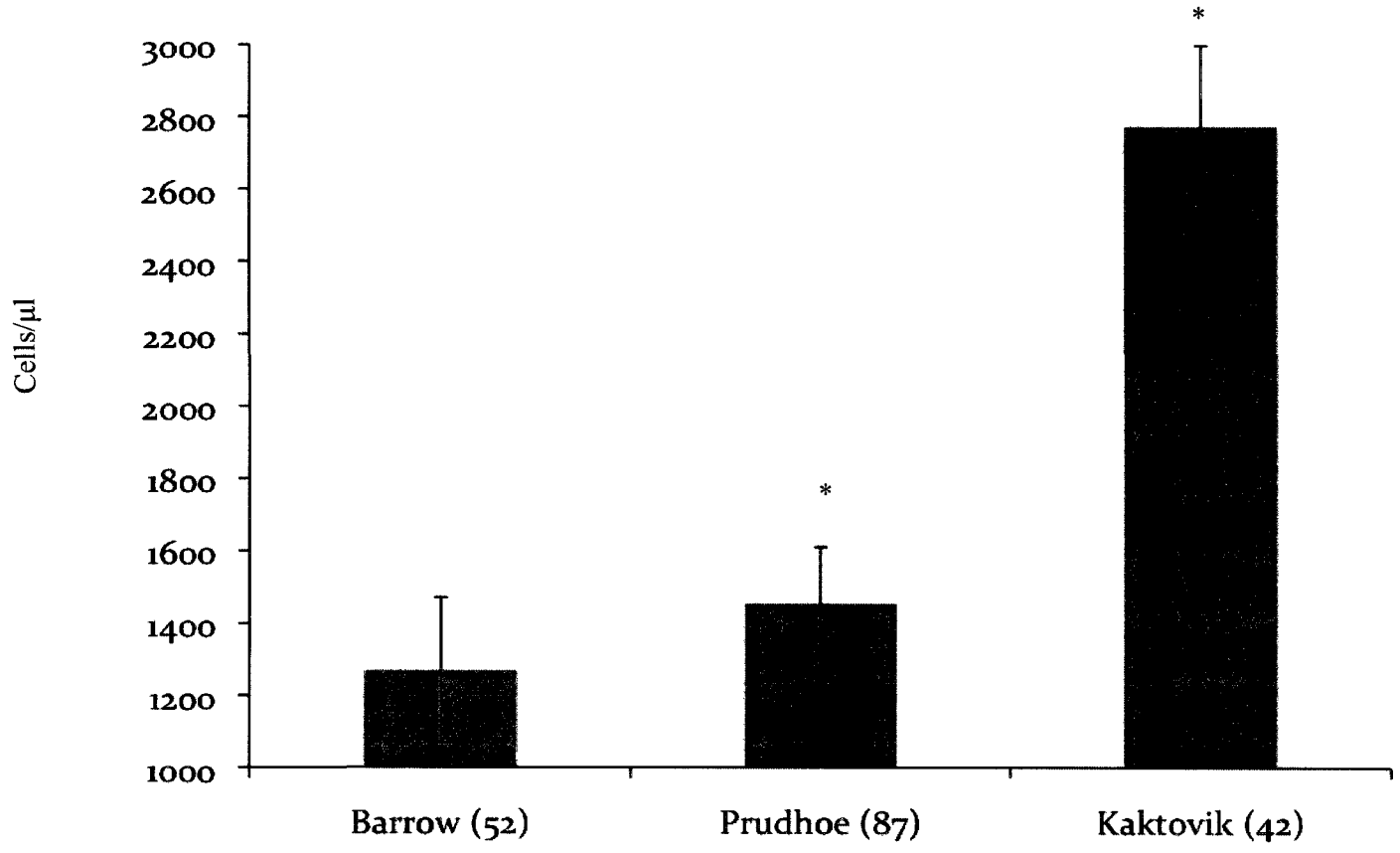

Figure 1.2 Mean of monocytes (± SE) by location (N) for southern Beaufort Sea, Alaska polar bears (2005-2007).

Table 1.1 (a) Hematology reference ranges for southern Beaufort Sea, Alaska polar bears for all cohorts and sample years combined; (b) hematology reference ranges for captive polar bears as cataloged in the International Species Database (ISIS) and for Western Churchill, Manitoba polar bears (Cattet and Caulkett, 2001); hematocrit values measured in springs 1982–1988 for southern Beaufort Sea polar bears (SBS) and for Manitoba polar bears (Lee et al., 1977).

(a)

	southern Beaufort Sea Polar Bears 2005-2007					
Parameter (unit)	Mean	Median	± SD	95% CI	N	Range
Leukocytes (/μl)	8130	7500	3200	7670-8594	187	2375-22625
Neutrophils (/μl)	5340	4640	2800	4935-5750	184	807-20457
Lymphocytes (/μl)	1528	1265	1039	1377-1679	184	184-7766
Monocytes (/μl)	17345	1105	1598	1502-1967	184	195-7642
Eosinophils (/μl)	399	349	333	350-447	184	0-1666
Basophils (/μl)	9	0	26	5-13	184	0-255
IgG (mg/ml)	24	21.8	9.3	22.7-25.4	178	9.5-48.9
Hematocrit (%)	44.9	45.5	4.9	44.2-45.6	189	21.5-61.5
Neutrophils (% of 100 cells)	64	66	14.2	62-66.1	181	10.8-90.4
Lymphocytes (% of 100 cells)	19.9	17.1	12	18.2-21.7	181	3-68
Monocytes (% of 100 cells)	10.8	9.8	5.4	10.1-11.6	181	3-36
Eosinophils (% of 100 cells)	5	4.8	3.6	4.5-5.5	181	0.3-20.5
Basophils (% of 100 cells)	0.1	0	0.3	0.1-0.2	181	0-2.0

Table 1.1 (Continued)

(b)

	ISIS				Cattet and Caulkett (2001)		SBS 1982-88				Lee et al. (1977)			
Parameter (unit)	Mean	± SD	Range	Sample # (N)	Range	N	Mean	± SD	Range	N	Mean	± SD	Range	N
Leukocytes (/µl)	9515	3801	2500-3300	249 (94)	3300-10800	18-151	ND	ND	ND	ND	ND	ND	ND	33
Neutrophils (/µl)	6555	2461	1930-2060	224 (90)	1955-6405	18-151	ND	ND	ND	ND	ND	ND	ND	33
Lymphocytes (/µl)	1628	820	296-5200	231 (90)	640-2095	18-151	ND	ND	ND	ND	ND	ND	ND	33
Monocytes (/µl)	534	377	0-2814	220 (84)	370-1210	18-151	ND	ND	ND	ND	ND	ND	ND	33
Eosinophils (/µl)	758	670	0-4238	217 (86)	130-420	18-151	ND	ND	ND	ND	ND	ND	ND	33
Basophils (/µl)	55	87	0-405	31 (20)	25-85	18-151	ND	ND	ND	ND	ND	ND	ND	33
IgG (mg/ml)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	33
Hematocrit (%)	44	5	30-59.8	258 (96)	36-53	18-151	45	4	ND	43	44.5	3.2	37-50	33
Neutrophils (% of 100 cells)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	33
Lymphocytes (% of 100 cells)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	33
Monocytes (% of 100 cells)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	33
Eosinophils (% of 100 cells)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	33
Basophils (% of 100 cells)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	33

Table 1.2 References ranges for southern Beaufort Sea, Alaska polar bears (2005-2007). By sex: (a) males (b) females (c) means for sexes compared.

(a)

	Males					
Parameter (unit)	Mean	Median	± SD	95% CI	N	Range
Leukocytes (/μl)	8872	7750	3085	7992-9365	80	3750-18625
Neutrophils (/μl)	5930	5199	2477	5268-6377	79	2200-15624
Lymphocytes (/μl)	1518	1313	925	1265-1679	79	315-5155
Monocytes (/μl)	991	865	514	878-1108	79	195-2850
Eosinophils (/μl)	479	413	364	407-570	79	26-1666
Basophils (/μl)	10	0	32	3-17	88	0-255
IgG (mg/ml)	27	25.5	9.9	24.4-28.9	75	10.3-48.9
Hematocrit (%)	47	47.0	4.4	46-48	81	31.5-61.5
Neutrophils (% of 100 cells)	65	66.0	11.1	63.1-68	79	29.5-86.5
Lymphocytes (% of 100 cells)	17.9	15.0	10.2	15.2-19.8	79	4.0-56.0
Monocytes (% of 100 cells)	11.1	10.6	4.3	10.3-12.2	79	3.8-25.0
Eosinophils (% of 100 cells)	5.5	5.3	3.5	4.8-6.4	79	0.3-20.5
Basophils (% of 100 cells)	0.1	0	0.3	0.1-0.2	79	0-2.0

Table 1.2 (Continued)

(b)

	Females					
Parameter (unit)	Mean	Median	± SD	95% CI	N	Range
Leukocytes (/µl)	7325	6750	2713	6754-7897	89	2375-15750
Neutrophils (/µl)	4690	4275	2213	4221-5158	88	807-12206
Lymphocytes (/µl)	1550	1207	1165	1303-1797	88	184-7766
Monocytes (/µl)	745	664	459	658-852	88	235-2670
Eosinophils (/µl)	333	255	297	258-384	88	0-1620
Basophils (/µl)	9	0	21	4-13	89	0-96
IgG (mg/ml)	21.1	20.1	7.6	19.4-22.7	86	9.5-48.4
Hematocrit (%)	43.4	44.4	4.2	42.5-44.2	91	33.3-51.8
Neutrophils (% of 100 cells)	62.9	66.3	15.7	60.1-66.6	90	10.8-89.0
Lymphocytes (% of 100 cells)	21.9	18.3	13	19-24.4	90	3.3-67.5
Monocytes (% of 100 cells)	10.6	9	6.1	9.3-12	90	4.3-36.4
Eosinophils (% of 100 cells)	4.5	3.3	3.3	3.5-4.9	90	0-17.3
Basophils (% of 100 cells)	0.1	0	0.3	0.1-0.2	90	0-1.5

Table 1.2 (Continued)

(c)

	Males	Females		
Parameter (unit)	Mean		t value; p-value	% Difference
Leukocytes (/μl)	8872	7325	3.62; 0.0004	21.1
Neutrophils (/μl)	5931	4741	3.62; 0.0004	25.1
Lymphocytes (/μl)	1519	1550	0.19; 0.8504	NA
Monocytes (/μl)	991	745	3.74; 0.0002	33
Eosinophils (/μl)	479	333	3.26; 0.0014	43.9
Basophils (/μl)	10	9	0.38; 0.7042	NA
IgG (mg/ml)	27	21.1	4.12; <0.0001	28
Hematocrit (%)	46.6	44.4	4.68;<0.0001	5
Neutrophils (% of 100 cells)	65.1	62.9	1.07; 0.8567	NA
Lymphocytes (% of 100 cells)	17.9	21.9	2.30; 0.0226	18.4
Monocytes (% of 100 cells)	11.1	10.6	0.72; 0.47	NA
Eosinophils (% of 100 cells)	5.5	4.5	1.81; 0.0734	21
Basophils (% of 100 cells)	0.1	0.1	0.25; 0.8066	NA

Table 1.3 Means compared for reproductive females ages ≥5 and males ages ≥5 compared to each female reproductive cohort.

	Reproductive females ages ≥5 compared				Males ages ≥5 compared to each female cohort			
	Lactating	Non-lactating			Males		Lactating	Non-lactating
Parameter (unit)	Mean		t value; p-value	% Difference	Mean	F-ratio; p-value	% Difference	
Leukocytes (/μl)	6042	8094	3.50; 0.0008	25.4	9023	10.05; <0.0001	49.3	NA
Neutrophils (/μl)	4427	5007	0.34; 0.7313	NA	6216	5.08; 0.0078	40.4	NA
Lymphocytes (/μl)	883	1801	4.52; <0.0001	51	1381	9.21; 0.0002	56.3	NA
Monocytes (/μl)	1156	1869	2.38; 0.0202	38.2	2175	8.12; 0.0002	88.1	NA
Eosinophils (/μl)	176	410	4.04; 0.0002	57	489	22.25;<0.0001	177.2	NA
Basophils (/μl)	3	12	1.72; 0.046	NA	11	NA	NA	NA
IgG (mg/ml)	20.6	23.4	0.82; 0.4136	10.8	28.2	5.62; 0.0046	28.5	NA
Hematocrit (%)	41.9	44.6	2.97; 0.0041	6.8	47.2	19.28;<0.0001	12.7	5.7
Neutrophils (% of 100 cells)	71.6	58.8	3.86; 0.0003	21.7	67	10.15; <0.0001	-6.8	13.5
Lymphocytes (% of 100 cells)	16	23.9	2.82; 0.0066	33	16	6.16; 0.0028	-33.0	NA
Monocytes (% of 100 cells)	9.4	11.9	1.93; 0.0595	21.5	11.3	3.53; 0.032	20.6	NA
Eosinophils (% of 100 cells)	3	5.2	2.58; 0.0121	42.4	5.5	11.22, <0.0001	83.1	NA
Basophils (% of 100 cells)	0.1	0.1	1.47; 0.1474	NA	0.1	NA	NA	NA

Table 1.4 References ranges for southern Beaufort Sea, Alaska polar bears (2005-2007) by cohort for ages 1-2, 3-4, 5-7, ≥ 8. (a) 1-2 years, (b) 3-4 years, (c) 5-7 years, (d) ≥ 8 years, (e) age cohort comparisons. NA=not applicable.

(a)

	1-2					
Parameter (unit)	Mean	Median	± SD	95% CI	N	Range
Leukocytes (/μl)	7946	7250	3398	6476-9415	23	3125-15750
Neutrophils (/μl)	5129	4010	3128	3777-6482	23	807-12206
Lymphocytes (/μl)	1885	1737	1019	1445-2326	23	569-5063
Monocytes (/μl)	1324	800	1377	728-1920	23	253-5298
Eosinophils (/μl)	252	245	154	185-319	23	33-709
Basophils (/μl)	6	0	16	0-13	23	0-61
IgG (mg/ml)	17.7	14.8	8.17	14.2-21.2	23	9.5-48.4
Hematocrit (%)	43.2	42.5	4.4	41.3-45.1	23	33.3-51.8
Neutrophils (% of 100 cells)	61.4	66	16.8	51.2-68.7	23	10.8-79.6
Lymphocytes (% of 100 cells)	26.2	24.5	14.6	19.9-32.5	23	8-67.5
Monocytes (% of 100 cells)	8.9	8.3	3.1	7.6-10.2	23	4.5-16.2
Eosinophils (% of 100 cells)	3.4	3.0	1.8	2.6-4.2	23	0.3-7.0
Basophils (% of 100 cells)	0.2	0	0.4	0-.3	23	0-1.5

(b) **Table 1.4 (Continued)**

	3-4					
Parameter (unit)	Mean	Median	± SD	95% CI	N	Range
Leukocytes (/µl)	8104	7000	3185	6407-9801	15	4250-13500
Neutrophils (/µl)	4345	3666	1938	3313-5378	15	1913-8322
Lymphocytes (/µl)	2219	1902	1458	1442-2996	15	478-5155
Monocytes (/µl)	1062	796	674	703-1421	15	279-2454
Eosinophils (/µl)	592	534	495	328-856	15	39-1666
Basophils (/µl)	14	0	26	1-28	15	0-64
IgG (mg/ml)	19.7	19.5	5.4	16.7-22.7	15	10.3-30.6
Neutrophils (% of 100 cells)	54.4	57	14.7	46.6-62.3	15	29.5-76
Lymphocytes (% of 100 cells)	26.4	22.9	12.4	19.8-33.0	15	8.5-56.0
Monocytes (% of 100 cells)	12.2	9.6	8.4	7.7-16.6	15	5.0-36.4
Eosinophils (% of 100 cells)	6.8	5.3	5.2	4.1-6.8	15	0.8-20.5
Basophils (% of 100 cells)	0.2	0	0.4	0-0.4	15	0-1.0

(c) **Table 1.4 (Continued)**

Parameter (unit)	5-7 Mean	Median	± SD	95% CI	N	Range
Leukocytes (/µl)	7319	7000	2709	6453-8186	38	2375-16875
Neutrophils (/µl)	4438	3987	2248	3720-5157	38	898-12353
Lymphocytes (/µl)	1690	1430	963	1382-1998	38	315-4140
Monocytes (/µl)	1759	940	1687	1220-1759	38	195-6581
Eosinophils (/µl)	389	360	326	285-493	38	26-1473
Basophils (/µl)	9	0	23	2-16	38	0-96
IgG (mg/ml)	21.6	21.4	6.9	23.8-29.3	38	11.9-38.6
Hematocrit (%)	48	45.6	4.2	43.7-46.4	38	34.5-55.8
Neutrophils (% of 100 cells)	59.6	61.1	14.3	55.1-64.2	38	33-84
Lymphocytes (% of 100 cells)	24	21.9	12	20-27.8	38	6.0-59.7
Monocytes (% of 100 cells)	10.4	9.1	5.3	8.7-12.1	38	4.0-34.3
Eosinophils (% of 100 cells)	5.3	5.0	4.3	3.9-6.6	38	0.5-18.7
Basophils (% of 100 cells)	0.1	0	0.3	0-0.2	38	0-1.1

(d) **Table 1.4 (Continued)**

	≥ 8					
Parameter (unit)	Mean	Median	± SD	95% CI	N	Range
Leukocytes (/µl)	8459	7750	3304	7803-9115	100	3375-22625
Neutrophils (/µl)	5886	5221	2841	5322-6449	100	1704-20457
Lymphocytes (/µl)	1265	1095	928	1081-1449	100	184-7766
Monocytes (/µl)	1861	1229	1607	1542-2180	100	235-7642
Eosinophils (/µl)	414	358	325	349-478	100	18-1614
Basophils (/µl)	10	0	30	3-16	100	0-255
IgG (mg/ml)	27.1	25.2	9.7	25.2-29	100	11.3-48.9
Hematocrit (%)	41.4	46	5	44.5-46.4	100	31.5-61.5
Neutrophils (% of 100 cells)	68.1	68	11.9	65.7-70.5	100	30.5-90.4
Lymphocytes (% of 100 cells)	15.6	13.0	9.4	13.7-17.4	100	3.3-57.0
Monocytes (% of 100 cells)	11.3	10.3	5.1	10.3-12.3	100	2.5-33.7
Eosinophils (% of 100 cells)	4.9	5.0	3.1	4.3-5.5	100	0.3-13.0
Basophils (% of 100 cells)	0.1	0	0.3	0.1-0.2	100	0-2.0

(e) **Table 1.4 (Continued)**

	1-2	3-4	5-7	≥ 8			
Parameter (unit)	Mean				Comparison	F ratio; p-value	% Difference
Leukocytes (/µl)	7946	8104	7319	8459	NA	1.51; 0.2121	NA
Neutrophils (/µl)	5129	4345	4438	5886	≥ 8 vs. 5-7	5.10; 0.0021	24.6
Lymphocytes (/µl)	1885	2219	1690	1265	≥ 8 vs.1-2, 3-4, 5-7	7.02; 0.0002	32.9, 43, 25.1
Monocytes (/µl)	1324	1062	1759	1861	NA	1.68; 0.1721	NA
Eosinophils (/µl)	252	592	389	414	NA	1.57; 0.1996	NA
Basophils (/µl)	6	14	9	10	NA	0.28; 0.8431	NA
IgG (mg/ml)	17.69	19.73	21.56	27.07	≥ 8 vs.1-2, 3-4, 5-7	10.48; <0.0001	53.02, 37.20, 25.56
Hematocrit (%)	43.2	45.3	48	41.4	NA	1.67; 0.1772	NA
Neutrophils (% of 100 cells)	61.4	54.4	59.6	68.1	≥ 8 vs. 3-4, 5-7	7.57; <0.0001	25.2, 14.2
Lymphocytes (% of 100 cells)	26.2	26.4	24	15.6	≥ 8 vs. 1-2, 3-4, 5-7	11.26; <0.0001	40.5, 41.0, 35
Monocytes (% of 100 cells)	8.9	12.2	10.4	11.3	NA	1.65; 0.1795	NA
Eosinophils (% of 100 cells)	3.4	6.8	5.3	4.9	3-4 vs. 1-2	3.19; 0.0252	101.5
Basophils (% of 100 cells)	0.2	0.2	0.1	0.1	NA	0.28; 0.8371	NA

CHAPTER 2

Morbillivirus and *Toxoplasma* exposure and association with hematological parameters for southern Beaufort Sea polar bears: Potential response to infectious agents in a sentinel species[1]

2.1 ABSTRACT

Arctic temperatures are increasing in response to greenhouse gas forcing and polar bears have already responded to changing conditions. Declines in body stature and vital rates have been linked to warming induced loss of sea-ice. As food webs change and human activities respond to a milder Arctic, exposure of polar bears and other arctic marine organisms to infectious agents may increase. Because of the polar bear's status as arctic ecosystem sentinel, polar bear health could provide an index of changing pathogen occurrence throughout the Arctic, however, exposure and monitoring protocols have yet to be established. We examine prevalence of antibodies to *Toxoplasma gondii,* and four morbilliviruses [canine distemper (CDV), phocine distemper (PDV), dolphin morbillivirus (DMV), porpoise morbillivirus (PMV)] including risk factors for exposure. We also examine the relationships between antibody levels and hematologic values established in the previous companion paper. Antibodies to *Toxoplasma gondii* and morbilliviruses were found in both sample years. We found a significant inverse

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relationship between CDV titer and total leukocytes, neutrophils, monocytes and eosinophils, and a significant positive relationship between eosinophils and *Toxoplasma gondii* antibodies. Morbilliviral prevalence varied significantly among age cohorts, with 1-2 year olds least likely to be seropositive and bears aged 5-7 most likely. Data suggest the presence of CDV and *Toxoplasma gondii* antibodies is associated with polar bear hematologic values. We conclude that exposure to CDV-like antigen is not randomly distributed among age classes and suggest that differing behaviors among life history stages may drive probability of specific antibody presence.

2.2 INTRODUCTION

Climate change has significant implications for the ecology of infectious disease in the Arctic. Evidence suggests that plant and animal range changes are occurring on a large scale in response to climate change. In a review by Root et al. (2003) including 143 studies of 1500 species, meta-analysis indicated 80% of species reviewed had shifted their ranges north. Shifts are likely to be greater in polar regions where change in temperature increase is greater. As species move into other regions they are likely to bring their pathogens with them (Bradley et al., 2005) thus compromising the health of the arctic ecosystem. Warmer temperatures and increased humidity may alter transmission patterns of existing infectious agents and introduce new diseases (e.g. alterations in vector ranges) previously unknown to the Arctic, and alter dynamics of existing disease reservoirs.

Species especially sensitive to such changes include ice-dependent top predators such as polar bears. As described in Kirk et al. (2010), due to intimate ties with sea-ice and adaptation to a relatively "disease free" environment, polar bears are likely to confront a variety of new challenges related to climate change. These challenges may enhance their susceptibility to new and increasing numbers of pathogens. As apex predators, polar bears can be used as sentinels of change in occurrence of infectious agents in the arctic food web and their potential transmission between the arctic marine, coastal and terrestrial environments.

Antibodies to morbillivirus and *Toxoplasma gondii* have been previously documented in polar bears throughout the Arctic (Cattet et al., 2004; Tryland et al., 2005; Garner et al., 2000; Zarnke et al., 2004; Rah et al., 2005; Oksanen et al., 2009; Tryland, 2000; Jensen et al., 2010). Transmission dynamics of morbilliviruses and *Toxoplasma* in the Arctic may be affected as climate changes impact vectors and the health, behavior, movement, and population dynamics of reservoir populations of marine (e.g. pinniped) and terrestrial hosts (e.g. domestic and wild canids). These agents have been associated with pathogen spillover to/from domestic species and with pathogen pollution between terrestrial and marine environments. Such phenomenona are anticipated to increase with warming in the Arctic (MacDonald et al., 2005). Furthermore, these agents have significant implications for human and wildlife health.

Because morbilliviruses and *T. gondii* pass between terrestrial and aquatic environments, they pose an important concern for marine organisms facing a warming world. For example CDV from terrestrial species (probably transmitted to seals from domestic dogs) was implicated in outbreaks among Baikal seals and Caspian seals (*Phoca caspica*, Kennedy et al., 2000). Conversely, PDV was suggested as the cause of distemper among farmed mink near coastal Denmark (Blixenkrone-Møller et al., 1989). Initial hypotheses suggested that exposure of polar bears to morbillivirus resulted from PDV infected seal prey. Pathogen pollution from terrestrial sources has been implicated for the presence of *T. gondii* in the marine environment (Miller et al., 2002). Since polar bears reside at the marine-terrestrial interface and have been increasingly "land-bound" in a decreasing sea

ice environment, they may serve as a link in the ecology of morbillivirus and *T. gondii* transmission in the Arctic ecosystem. With changes in sea-ice extent and in the chronology of melting and reformation, polar bears are likely to spend more time onshore in proximity to expanding human habitation and industrialization and in contact with species with which they formerly had limited or no interaction. Groups of animals [including polar and grizzly (*Ursus arctos*)] bears congregate at hunter killed whale carcasses on the outskirts of some villages (Bentzen et al., 2008). Terrestrial based food sources, including those of human origin are likely to become of greater importance with changes in sea-ice conditions. These food sources are potential reservoirs of infectious agents including morbillivirus and *T. gondii*.

A hallmark of morbillivirus is the ability to interfere with specialization and differentiation of lymphocytes (Heaney et al., 2002). Immune suppression associated with morbillivirus infection or exposure can influence the mortality rate of infected individuals by facilitating secondary infections or activating latent infections, which can be lethal to the host. Although not zoonotic, morbillivirus has potential to significantly impact populations of marine mammals important to subsistence hunters. These foods provide an important source of protein and fatty acids and for some users, are the main source of these nutrients. *T. gondii*, in contrast, is typically of little clinical significance in immunocompetent animals, yet severe disease can result from latent infection in the presence of other stressors, which could include morbilliviral infection (Dubey et al., 1989). Clinical toxoplasmosis has been diagnosed during necropsy evaluations of seals,

dolphins and beluga whales confirming the possibility of natural transmission in the marine environment (Tryland, 2000). *T. gondii* is zoonotic and is of documented public health significance. Humans and wildlife become infected with *T. gondii* by ingesting raw or insufficiently cooked meat containing bradyzoites, ingesting oocysts from cat feces directly or indirectly via soil or other items that have been in contact with oocysts, or through vertical transmission from a mother to her fetus (Gilbertson et al., 2005). These transmission modes are likely to increase in a warming Arctic. As in wildlife, this parasite typically significantly impacts only the health of immunocompromised individuals, however, it is well documented that infants infected *in utero* may develop learning and visual disabilities later in life (Gilbertson et al., 2005).

Introduction of anthropogenic agents, alterations in pathogen transmission pathways and foraging behavior may expose polar bears to an increased variety of infections to which they lack immunity. Here, we establish seroprevalence patterns for exposure risk and associations with hematological biomarkers for morbillivirus and *Toxoplasma gondii* in southern Beaufort Sea polar bears. Changes in morbillivirus and *T. gondii* prevalence that may occur with climate change could pose an increased threat to the health of local people living along the coast, trappers and wildlife. By establishing seroprevalence patterns for two indicator infectious agents (morbillivirus and *T. gondii*) and by drawing associations between seroprevalence and hematological biomarkers described in our companion paper, we demonstrate how monitoring polar bear health could provide an index of changing pathogen occurrence throughout the Arctic.

2.3 METHODS

Methods for capture including location, blood sample processing, hematology and cohort designations are described in Kirk et al. (2010). Blood was collected from the femoral vein or artery (Vacutainer, BD Biosciences; Franklin Lanes, NJ) into evacuated blood collection tubes for whole blood and serum and PAXgene Blood RNA tubes (Qiagen, Valencia, California) for RNA isolation. Viral CULTURETTE™ Collection and Transport Systems (BD Biosciences; Franklin Lanes, NJ) were also used to swab nasal cavities for morbilliviral RNA detection. Although we evaluated hematological values in southern Beaufort Sea polar bears captured over a three-year period (2005-2007), we examined specific antibody indicators in 2005 and 2006 only.

2.3.1 Serology

Serum was screened for morbilliviral and *T. gondii* antibodies at the Oklahoma State University Animal Disease Diagnostic Laboratory (Stillwater, OK USA). Differential serum neutralization (SN) assay for four morbilliviruses (CDV, DMV, PDV, PMV) was performed, as described by Garner et al. (2000). Results are expressed as the reciprocal of the highest dilution that completely neutralized 100% of the respective challenging virus ('neutralized'), using a cut-off of 1:4. Latex agglutination assay was performed for *T. gondii* as described previously by Rodgers and Baldwin (1990). Results are expressed as the reciprocal of the highest dilution of serum resulting in a clear agglutination; titers >1:16 were considered positive.

2.3.2 Molecular Analyses

RNA was extracted from blood using the PAXgene Blood RNA kit (Qiagen, Valencia, California) and nasal swabs using TRI REAGENT® LS (Molecular Research Center, Inc., Cincinnati, OH); according to the manufacturers' instructions. Sample integrity was assessed via amplification of a housekeeping gene, β-actin (Kraft et al., 1995). We used the universal primer set, which amplifies a well-conserved region of the polymerase cofactor, phophoprotein for all known morbilliviruses (Barrett et al., 1993). Reverse transcription reactions were performed using SuperScript™ III One-Step RT-PCR System with Platinum® Taq, according to the manufacturer's instructions (Invitrogen, Carlsbad, California).

2.3.3 Statistical Analyses

We calculated summary statistics for all parameters using JMP 7 statistical software (SAS Institute, Cary, NC, USA). We assessed normality in the distributions of outcome values with Shapiro-Wilk goodness of fit test. Log transformations were employed where data were not normally distributed. Statistical analyses were performed using log transformed titer data, while means and ranges were calculated using raw titer data (1/titer). We used Pearson's correlations to evaluate bivariate relationships among hematologic endpoints for 2005 and 2006 independently, and years combined (JMP 7; SAS Institute, Cary, N.C., USA). For parameter pairs where correlations were significant (e.g. CDV titer and total leukocyte count), the difference between the mean of a given parameter for all CDV positive animals and CDV negative animals was determined. The

resulting number was divided by the mean for that parameter for all CDV negative animals and this number was converted to a percentage yielding "percentage mean difference" (Kirk et al., 2010).

We compared mean CDV titers for seropositive bears in each age class using ANOVA and the post-hoc Tukey-Kramer test. Risk factor analyses were conducted for the presence of antibodies to morbillivirus and *T. gondii* using statistical package R with epitools and epicalc libraries (R Development Core Team, 2006). We used χ^2 analysis (function prop.test) to test for homogeneity in prevalence between sampling years, sex, and age class. Recognizing that potential exposure differed at each of our logistics bases (Barrow, Prudhoe Bay, Kaktovik), we also used χ^2 analysis to test whether prevalence was homogeneous across logistics bases. The Cochran-Mantel Haenszel χ^2 test evaluated year-to-year and between sex differences in prevalence stratified by age (function mantelhaen.test). Probability of exposure to CDV-like antigen or *T. gondii* was assessed by age cohort using odds ratios (OR) and 95% confidence intervals (function ci.binomial), comparing bears aged 3-4, 5-7 and ≥8 years of age to bears aged 1-2. Interaction between the variables; age, sex, and year upon risk for presence of CDV antibodies were explored via logistic regression [function glm (CDV.sero ~ age + sex + yearf, family=binomial)] utilizing a backwards stepwise approach and Akaike's Information Criteria for model selection (functions step, update, logistic.display). The variable CDV.sero indicates whether the animal was seropositive or seronegative, age is a continuous variable, sex is male or female and year is 2005 or 2006. We did not search

for interactions affecting risk for presence of *T. gondii* antibodies because there was no significant difference in prevalence among cohorts. In 2006, we were able to recapture 6 antibody positive individuals originally captured in 2005. This allowed us to assess possible changes in prevalence and titer calculations over time.

2.4 RESULTS

We analyzed samples from 136 polar bears: 20 1-2, 12 3-4, 14 5-7, and 84 bears ≥8 years, ranging up to 24 years of age. Mean values for total leukocytes, neutrophils, lymphocytes and monocytes measured in the springs of 2005 (n=61) and 2006 (n=67) for all cohorts combined were within one standard deviation of values reported for captive polar bears (ISIS) (Figure 2.1). Seroprevalence of CDV in both 2005 and 2006 was relatively high (Table 2.1). Antibodies to PDV, DMV and PMV and *T. gondii* were also detected in both sample years (Table 2.1). Mean titers for seropositive polar bears were highest for CDV (titer range = 1:8-1:512), followed by PDV (titer range = 1:8-1:96) DMV (titer range = 1:8-1:24) and lowest for PMV (titer range = 1:16-1:32) as indicated in Figure 2.2. Each animal seropositive for DMV, PDV, and/or PMV presented a higher titer to CDV (Figure 2.3). Of all polar bears seropositive for CDV, 48.5% were also positive for PDV. Any animal positive for DMV or PMV had antibodies to both CDV and PDV (Figure 2.4). Three polar bears were seropositive for all 4 viruses. Of polar bears seropositive in 2005, we recaptured 6 in 2006. All 6 animals remained seropositive and in 4 of these animals, titers decreased by one dilution factor. One animal experienced an increase in titer by one dilution factor from 1:48 to 1:64 and another's titer remained

the same. The proportion of polar bears seropositive for *T. gondii* was not significantly different between age class or sex with positive titers ranging from 1:16-1:2048. Viral RNA was not detected in blood samples or nasal swabs.

2.4.1 Hematology and Serology Associations

We examined relationships between select infectious agent antibody titers and hematological parameters revealing significant decreases in neutrophil, eosinophil, monocyte, and total leukocyte counts with increasing CDV titer (Table 2.2). Relationships were statistically significant in 2005 and for combined sample years; no significant associations were found when data were analyzed for 2006 alone. We also detected a significant positive relationship between eosinophil count and *T. gondii* titer in 2005 but not in 2006 or when data were pooled across sample years. No difference was demonstrated in titers by age class or sex and no associations were found for lymphocytes or basophils and infectious agent titer.

2.4.2 Risk Factors for Morbillivirus and *Toxoplasma gondii* Exposure

Homogeneity tests indicated prevalence to CDV varied significantly ($p = 0.001$) among age cohort while prevalence to *T. gondii* did not. Polar bears 1-2 years of age were least likely to be seropositive for CDV antibodies and 5-7 year olds were most likely to be seropositive. Polar bears aged 3-4 were approximately equally likely to possess CDV antibodies as 1-2 year olds and bears ≥8 years of age were approximately five times as likely to be seropositive. Variation between years in CDV prevalence among age cohorts

was significant ($\chi 2 = 16.244$, df = 4, p = 0.003; Table 2.1c) using the Cochran-Mantel-Haenszel test. The percentage of bears seropositive for CDV at each individual age is shown in Figure 2.2. We did not observe a difference in CDV antibody prevalence between males and females in 2005, 2006 or for sample years combined. This absence of an obvious link between sex and seropositivity was evident in a logistic regression of sex, age and year. That analysis revealed that the likelihood of having antibodies varied according to interaction of year and sex (sex*year: OR = 5.30, p = 0.029) with bears approximately 5 times as likely to be seropositive if they were female in 2006 or male in 2005. Such a "flip-flop" pattern would not be expected if seropositivity were strongly sex linked. There was no difference in the proportion of bears seropositive for CDV or *T. gondii* between logistic base locations for sexes combined or between sexes.

2.5 DISCUSSION

The high prevalence of antibodies we observed to four morbilliviruses was consistent across sample years and with previous studies conducted for Alaska polar bears (Garner et al., 2000). Serum neutralization assay results indicate that the virus(es) to which northern Alaskan polar bears have been exposed is most antigenically related to CDV. Current findings are consistent with that of Garner et al. (2000) who concluded morbillivirus exposure in Alaska polar bears was most likely of terrestrial origin because each individual positive for either PDV, DMV, or PMV presented a higher titer (more reactive) to CDV. However, using these serological assays, we cannot determine whether

these bears are infected with or exposed to a single strain of morbillivirus, multiple morbilliviruses or perhaps carry a distinct strain enzootic to polar bears.

Polar bears seropositive for CDV-like viruses have been found across the Arctic. In portions of Canada and in Svalbard, prevalence has been documented at 24% and 8%, respectively (Cattet et al., 2004; Tryland et al., 2005). In Alaska and Russia (Bering, Chukchi, and East Siberian seas) CDV prevalence has ranged from 26-46% depending upon sample year (Follmann et al., 1996). Morbilliviral antibodies have also been detected in terrestrial Arctic-dwelling carnivores in Alaska including: grizzly bears (Chomel et al., 1998; Philippa et al., 2004), and wolves (*Canis lupus*) (Zarnke et al., 2004). Evidence of CDV in arctic fox (*Alopex lagopus*) has been mostly anecdotal, however they are hypothesized to have been the source of infection for distemper outbreaks among sled dogs in the Arctic (Bohm et al., 1989; Campbell et al., 2007). Antibodies to morbilliviruses have not been documented in Alaskan Arctic marine mammals other than polar bears, however they have been reported in various pinniped and cetacean species in the Canadian Arctic including ringed seals (*Phoca hispida*), the polar bear's main prey (Duignan et al., 1997).

2.5.1 Hematology and Serology Associations

The high prevalence indicates a negligible mortality rate (large percentage of bears apparently recover) from infection or exposure (e.g. presence of antibody does not equate to infection) to morbillivirus in polar bears. However, changes observed in hematological

parameters are suggestive of a biologically significant response. The significant inverse relationship between these parameters indicates the virus may have an immunosuppressive effect or alternatively that immuno-suppressed individuals are more likely to be seropositive to the virus. We recognize reported r-values are low, however this is likely due to the contribution of additional variables not accounted for in the model and/or some nonlinearity in the observed relationship. That a significant inverse relationship was observed in 2005 but not 2006 may reflect natural fluctuation in the population in response to changes in infectious agent exposure and/or immunity.

While we observed significant hematological associations with CDV status in polar bears, we do not know if seropositive animals are adversely affected by and/or susceptible to morbilliviral infection. We did not observe overt clinical signs typically associated with the disease in domestic or wildlife species upon gross examination of bears captured in this study and we did not identify viral RNA in blood and nasal swab samples. In order for virus to be detected, bears needed to be sampled during active viremia. Possibly, antibody production results from exposure only, with no significant resulting viremia or a viremia of no consequence. Alternatively, the virus may be able to replicate within polar bears without adverse effects, yet serve as a reservoir for other sympatric carnivores. This phenomenon has been observed among some seal species (Duignan et al., 1997).

The inverse relationship between morbillivirus antibody titer and leukocytes observed in this study is consistent with observations documented in other species. In domestic canines and some wildlife species, CDV infection is characterized by immunosuppression followed by secondary infection. Much *in vitro* and *in vivo* work has been done exploring the pathogenesis of CDV using gnotobiotic canines in controlled laboratory studies. For example, viral antigen in monocytes (Appel and Gillespie, 1972) and circulating B and T lymphocytes (Krakowa et al., 1975) occurs during the acute phase of CDV infection. In dogs recovering from the disease, the presence of virus in the bloodstream is transient, however alterations in the anatomy of lymphoid tissue result (Krakowa et al., 1980). A similar pattern of infection in polar bears could account for the high percentage of bears found antibody positive without virus being detected. *In vitro* studies using lymphocytes isolated from convalescent nonviremic dogs exhibit a decreased response to mitogens when challenged (Krakowa, 1982). Furthermore, Krakowa et al. (1987) discovered that infection with CDV results in both virus-dependent and independent immunosuppression. For instance, when lymphocytes from viremic dogs were cultured together with lymphocytes isolated from uninfected dogs, lymphocytes from CDV infected dogs suppressed the phytomitogen responses of uninfected responder dogs. Perhaps more importantly, lymphocytes collected from convalescent, seropositive dogs that were no longer viremic suppressed responses of uninfected responder dogs. These findings have significant implications. They demonstrate that although animals that survive and clear infection may be protected from repeated infection, they may be

immunologically compromised and therefore more susceptible to other opportunistic and non-opportunistic infections.

Over the past two decades, these closely related morbilliviruses have caused significant disease and mortality in several marine and terrestrial mammal species (e.g. Dietz et al., 1989; Aguilar and Raga, 1993; Kennedy et al., 2000). In some mortality events attributed to morbilliviral infections among seals and dolphins, which like polar bears are top predators in their systems, infected animals were also exposed to high levels of persistent lipophilic environmental contaminants accumulated through the food chain (De Swart et al., 1996). This observation led to the hypothesis that contaminant related immunosuppression may have contributed to the clinical severity of the outbreaks. Polar bears bioaccumulate lipophilic compounds such as highly persistent organochlorines (OCs) (De Wit et al., 2002). Measurements of OCs in northern Alaska polar bears indicate a varied geographical distribution and relatively low concentrations (Bentzen et al., 2008). Despite low concentrations, the effects of contaminants on polar bears are of particular concern due to some unique physiological features potentially rendering them more vulnerable to the adverse effects of OCs. These include dramatic periods of fat accumulation followed by extended periods of fasting, delayed implantation, and unique mechanisms of biotransformation which can result in the accumulation of different mixes of parent compounds and metabolites as compared to other species (Fisk et al., 2005). Studies on Svalbard polar bears demonstrated negative correlations between PCBs (a

type of OC) and antibody-mediated immunity (Bernhoft et al., 2000; Lie et al., 2004). Thus morbillivirus may not act independently on the immune system.

We also observed a positive relationship between eosinophil count and *T. gondii* antibody titer with a maximum titer occurring in one individual of 1:1024. Eosinophils are a type of leukocyte characteristically released in response to parasitic infections and allergens, observed results are therefore, indicative of an expected response. Toxoplasmosis has been demonstrated to cause a low and discontinuous eosinophilia (Ripert, 2000). The apparent relationship between eosinophils and *T. gondii* antibody titer is suggestive of ongoing exposure to *T. gondii* rather than a previously resolved infection or exposure. *T. gondii* is typically of little clinical significance in immunocompetent animals and humans unless contracted during pregnancy. Severe clinical disease could however develop from a latent infection as immunity declines with age or where concurrent infectious or toxicant immunosuppressive stressors occur (Dubey et al., 1989).

2.5.2 Risk Factors for Morbillivirus and *Toxoplasma gondii* Exposure

Results of risk factor analyses for morbillivirus indicate a significant increase in prevalence with age peaking or reaching a plateau at sexual maturity. CDV prevalence was lowest in 1-2 year olds and greatest in 5-7 year olds for both sexes at all sampling locations. Polar bears are mostly solitary, interacting predominantly during the mating season or as female-cub(s) groups; therefore the time at which a polar bear terminates its juvenile period and becomes sexually active is analogous to a "first contact" setting in

which an immunologically naïve host is exposed to an increased variety of infectious agents. Sexually mature polar bears range widely during the breeding season and higher prevalence among prime aged bears could reflect increased probability of interacting with sources of exposure by associating with numerous different bears through breeding and fighting between males and possible alterations in foraging patterns. However, whether this observed pattern for risk arises from cumulative exposure to CDV-like antigen over time or from age-related changes in susceptibility remains to be determined. For example, once exposed and antibodies are generated, the animal may be more immunologically responsive in subsequent exposures. A more comprehensive understanding of CDV epidemiology, including both roles and interactions of demographic, spatial, and temporal factors, is needed to assess the implications of morbilliviral exposure in southern Beaufort Sea polar bears.

Our analyses of risk factors for the presence of antibodies to *T. gondii* indicate that age, sex, and geographic area do not affect the likelihood of exposure to this pathogen for southern Beaufort Sea polar bears in Alaska. However, the total number of animals we sampled and found seropositive for both sample years combined was relatively low (13.2%), limiting the power of this assessment. Although incidence of clinical toxoplasmosis among marine mammals appears low, antibodies to the parasite have been detected in numerous species across the Arctic (Tryland, 2000). Seroprevalence for Alaskan polar bears was 6% (Rah et al., 2005) and in Svalbard and Greenland polar bears ranged from 3.6%-28.7% depending upon age, sex, and location (Oksanen et al., 2009).

Antibodies to *T. gondii* occur in several species sharing habitat with polar bears in Alaska including: walrus (*Obobenus rosmarus*), bearded seals (*Erignathus barbatus*), spotted seals (*Phoca largha*) and ringed seals (Dubey et al., 2003); grizzly bears (Zarnke et al., 2000); wolves, and caribou (*Rangifer tarandus*) (Zarnke et al., 1997) and in Canada: hooded (*Cystophora cristata*) and grey seals (*Halichoerus grypus*) (Measures et al., 2004). Antibodies have also been detected in wildlife species in other regions of the state including black bears (*Ursus americanus*), harbor seals (*Phoca vitulina*), stellar sea lions (*Eumetopias jubatus*) and in one sea otter in southcentral Alaska (Zarnke et al., 2000; Gilbertson et al., 2005). Seropositive lynx (*Lynx canadensis*) have been found in interior Alaska and are the only wildlife species in the state known to pass *T. gondii* oocysts in feces (Zarnke et al., 2001). Antibodies were not detected in a serosurvey of 645 arctic marine mammals [harp (*Phoca groenlandica*), hooded ringed seals, and minke whales (*Balaenoptera acutorostrata*)] from the coast of Norway and the Barents Sea (Oksanen et al., 1999), however prevalence for *T. gondii* in arctic fox on Svalbard was 43% (Prestrud et al., 2007).

Serosurveys of other arctic wildlife on the north coast of Alaska such as arctic fox, ice seals (polar bear prey) and walrus as well as domestic dogs (*Canis familiaris*) could assist in elucidating whether sources of morbillivirus and *T. gondii* exposure for polar bears in Alaska are of marine or terrestrial origin, as they represent potential reservoirs for these agents. If morbilliviral nucleic acid were isolated from polar bears, sequence data would provide valuable information regarding probable sources and transmission pathways.

There are numerous potential routes for morbillivirus and *T. gondii* transmission, which may be impacted by warming in the Arctic and there is reason for concern that exposure may increase with such changes. This may occur for example, as potential reservoir species such lynx (*T. gondii*) and other carnivores (*T. gondii* and morbillivirus) move further north and as polar bears are increasingly forced to spend more time on land. A serosurvey of grizzly bears in Alaska demonstrated higher prevalence of *T. gondii* in northern Arctic regions than in other regions of Alaska (Zarnke et al., 1997). Because this region is where grizzly bear habitat overlaps with that of polar bears, it is reasonable to conclude that contact between polar bears and grizzly bears may increase. Changes in carnivory may occur as species access alternate food, sources driven by climate change. Cannibalism, which has been observed among polar bear (Amstrup et al., 2006), arctic fox and walrus, is a suspected means for the maintenance of *T. gondii* in a population (Prestrud et al., 2007). Scavenging on carcasses is also a known means for morbillivirus transmission and survival of the virus in the environment may improve in a warmer, wetter Arctic. The infectivity of *T. gondii* is enhanced by the aeration and humidity of soil conditions (Dubey, 2004). Increasing temperatures and humidity can thus result in increasing transmission of *T. gondii* with dissemination of fewer eggs from definitive hosts. Increasing sea levels and coastal erosion have the potential to increase pathogen pollution into the marine environment. Thawing of tundra combined with increased precipitation may mobilize pathogens from sewage lagoons and solid waste dumps containing human and pet waste in Arctic communities. With permafrost degradation, landfills can be washed directly into rivers or the ocean (MacDonald et al., 2005).

Pathogen pollution from terrestrial to the marine environment is a common phenomenon in temperate areas. For example, coastal freshwater surface runoff was determined to be a risk factor for sea otter (*Enhydra lutis*) infection with *T. gondii* in California (Miller et al., 2002). Studies in Svalbard revealed that the prevalence of *T. gondii* in polar bears has doubled in the past decade and now antibodies have been found in ringed seals for the first time. Jensen et al. (2010) hypothesize that survivorship of oocysts transported via the North Atlantic current from southern latitudes may have increased due to warming waters and the concomitant influx of marine invertebrate filter feeders that may serve as vectors for oocysts. Jensen et al. (2010) point out that the recent change in species diversity of migratory birds in the area, as well as increased human traffic, may also be responsible for observed increases. Alterations in avian migration routes with climate change, can similarly impact the ecology of *T. gondii* in northern Alaska.

2.6 CONCLUSION

Arctic marine ecosystem health is likely to be disrupted by a warming climate and the associated loss of essential sea ice habitats. Polar bears reside at the marine-terrestrial interface and may be increasingly exposed to infectious agents from multiple sources. A warming Arctic may alter microbial and parasite transmission pathways as well as susceptibility of polar bears to disease. Currently, limited data exist on the health status of U.S. polar bear populations. The current effects of morbillivirus on polar bear health are unclear. However, the combination of low-level effects of morbillivirus and *T. gondii* exposure with potential increases in nutritional stress due to losses of essential hunting

habitats may have significant implications for polar bear health and population dynamics. Our findings underscore the importance of efforts to monitor southern Beaufort Sea polar bears on a physiological as well as population level, and provide a benchmark for hematological parameters as well as seroprevalence and risk factors for "indicator" pathogens. Associations we develop between serological evidence of exposure to infectious agents and hematological parameters described in our companion paper substantiate the value of using these sentinel species biomarkers to monitor Arctic ecosystem health. The hematology and serology data we present for Alaskan polar bears emphasize the potential importance of synergisms among various potential stressors (morbillivirus, organochlorines, climate change, increased human presence etc.) that may undermine the future resilience (immune system) of polar bears to changes in their habitats. The sentinel position of polar bears, and the influence of their essential sea-ice habitats on global climate suggest that monitoring these indicators in polar bears has ramifications throughout the Arctic marine ecosystem and beyond.

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2.8 REFERENCES

Aguilar A, Raga J. 1993. The striped dolphin epizootic in the Mediterranean Sea. Ambio 22(8):24–528.

Amstrup SC, Stirling I, Smith TS, Perham C, Thiemann GW. 2006. Recent observations of intraspecific predation and cannibalism among polar bears in the southern Beaufort Sea. Polar Biology 29(5):997-1002.

Appel MJ, Gillespie JG. 1972. Canine distemper virus. In: Gard S, Hallauer C, Meyer KF (editors), Virology Monographs, Vol. 11. New York, NY: Springer-Verlag.

Barrett T, Visser IKG, Mamaev L, Van Bressem MF, Osterhaus ADME. 1993. Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology 193(2):1010-1012.

Bentzen TW, Follmann EH, Amstrup SC, York GS, Wooler MJ, Muir DCG, O'Hara TM. 2008. Dietary biomagnification of organochlorine contaminants in Alaskan polar bears. Canadian Journal of Zoology 86(3):177-191.

Bernhoft A, Skaare JU, Wiig Ø, Derocher AE, Larsen HJS. 2000. Possible immunotoxic effects of organochlorines in polar bears (*Ursus maritimus*) at Svalbard. Toxicology and Environmental Health 59(7):561–574.

Bohm J, Blixenkrone-Møller M, Lund E. 1989. A serious outbreak of canine distemper among sled-dogs in northern Greenland. Arctic Medical Research 48(4):195-203.

Bradley MJ, Kutz SJ, Jenkin E, O'Hara TM. 2005. The potential impact of climate change on infectious diseases of Arctic fauna. International Journal of Circumpolar Health 64(5):468-478.

Campbell D, Welch K, Baker C, Overell J. 2007. Canine distemper virus in sled dogs-Nunavut and NT. Canadian Cooperative Wildlife Health Centre Newsletter 13(1):8-9.

Cattet MRL, Duignan PJ, House CA, St. Aubin, DJ. 2004. Antibodies to canine distemper and phocine distemper viruses in polar bears from the Canadian Arctic. Journal of Wildlife Diseases 40(2):338–342.

Chomel BB, Kasten RW Chappuis G, Soulier M, Kikuchi Y. 1998. Serological survey of selected canine viral pathogens and zoonoses in grizzly bears (*Ursus arctos horribilis*) and black bears (*Ursus americanus*) from Alaska. Revue Scientifique et Technique 17(3):756-766.

De Swart RL, Ross PS, Vos JG, Osterhaus AD. 1996. Impaired immunity in harbour seals (*Phoca vitulina*) fed environmentally contaminated herring. Veterinary Quarterly 18(3):S127-128.

De Wit CA, Fisk AT, Hobbs KE, Muir DC, Gabrielsen GW, Kallenborn R, Krahn M, Norstrom RJ, Skaare JU (editors). 2004. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic, Oslo, Norway: Arctic Monitoring and Assessment Programme (AMAP).

Dietz R, Ansen CT, Have P, Heide-Jorgensen MP. 1989. Clue to seal epizootic? Nature 338(6217):627.

Dubey JP, Carpenter JL, Topper MJ, Uggla A. 1989. Fatal toxoplasmosis in dogs. Journal of the American Animal Hospital Association 26(6):659–664.

Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, Davis JW, Ewing R, Mense M, Kwok OC, Romand S, Thulliez P. 2003. *Toxoplasma gondii, Neospora caninum, Sarcocystis neurona*, and *Sarcocystis* canis-like infections in marine mammals. Veterinary Parasitology 116(4):275-296.

Dubey JP. 2004. Toxoplasmosis - a waterborne zoonosis. Veterinary Parasitology 126(1-2):57-72.

Duignan PJ, Nielsen O, House C, Kovacs KM, Duffy N, Early G, Sadove S, St. Aubin DJ, Rima K, Geraci JR. 1997. Epizootiology of morbillivirus in harp, hooded, and ringed seals from the Canadian arctic and western Atlantic. Journal of Wildlife Diseases 33(1):7-19.

Fisk AT, De Wit CA, Wayland M, Kuzyk ZZ, Burgess N, Letcher R, Braune B, Norstrom R, Blum SP, Sandau C, Li E, Larsen HJS, Skaare JU, Muir DCG. 2005. An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. Science of the Total Environment 351-352:57-93.

Follmann EH, Garner GW, Evermann JF. 1996. Serological evidence of morbillivirus infection in polar bears (*Ursus maritimus*) from Alaska and Russia. The Veterinary Record 138(25):615-618.

Garner GW, Evermann JF, Saliki JT, Follmann EH, McKeirnan AJ. 2000. Morbillivirus ecology in polar bears (*Ursus maritimus*). Polar Biology 23(7):474-478.

Gilbertson J, Mandsagar R, Funk. 2005. Preventing Infection with *Toxoplasma gondii*. State of Alaska Epidemiology Bulletin Number 7.

Heaney J, Barrett T, Cosby SL. 2002. Inhibition of *in vitro* leukocyte proliferation by morbilliviruses. Journal of Virology 76(7):3579–3584.

Jensen SK, Aars Jon, Lydersen C, Kovacs KM, Åsbakk K. 2010. The prevalence of *Toxoplasma gondii* in polar bears and their marine mammal prey: evidence for a marine transmission pathway? Polar Biology 33(5):599-606.

Kennedy S, Kuiken T, Jepson PD, Deaville R, Forsyth M, Barrett T, van de Bildt MW, Osterhaus AD, Eybatov T, Duck C, Kydyrmanov A, Mitrofanov I, Wilson S. 2000. Mass die-off of Caspian seals caused by canine distemper virus. Emerging Infectious Diseases 6(6):637-639.

Kirk CM, Amstrup S, Swor R, Holcomb D, O'Hara TM. 2010. Hematology of southern Beaufort Sea polar bears (2005-2007): Biomarker for an Arctic ecosystem health sentinel. EcoHealth doi:10.1007/S10393-010-0322 [Online July 7, 2010].

Kraft AE, Lichy JH, Lipscomb TP, Klaunberg BA, Kennedy S, Taubenberger JK. 1995. Postmortem diagnosis of morbillivirus infection in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic and Gulf of Mexico epizootics by a polymerase chain reaction based assay. Journal of Wildlife Disease 31(3):410-415.

Krakowa S, Cockerell G, Koestner A. 1975. Effects of canine distemper virus infection on lymphoid function *in vitro* and *in vivo*. Infectious Immunology 11(5):1069-1078.

Krakowa S, Higgins RJ, Koestner A. 1980. Canine: Review of structural and functional modulations in lymphoid tissue. American Journal of Veterinary Research 41(26):284-292.

Krakowa S. 1982. Mechanisms of *in vitro* immunosuppression in canine distemper virus infection. Journal of Clinical Laboratory Immunology 8(3):187-196.

Krakowka S, Ringler SS, Lewis M, Olsen RG, Axthelm MK. 1987. Immunosuppression by canine distemper virus: modulation of in vitro immunoglobulin synthesis, interleukin release and prostaglandin E2 production. Veterinary Immunology and Immunopathology 15(3):181-201.

Lie E, Larsen HJS, Larsen S, Johansen GM, Derocher AE, Lunn NJ, Norstrom RJ, Wiig Ø, Skaare JU. 2004. Does high organochlorine (OC) exposure impair the resistance to infection in polar bears (*Ursus maritimus*)? Part I: Effect of OCs on the humoral immunity. Toxicology and Environmental Health 67(7):555-582.

MacDonald RW, Harner T, Fyfe J. 2005. Recent climate change in the Arctic and its impact on contaminant pathways and interpretation of temporal trend data. Science of the Total Environment 342(1-3):5-86.

Measures LN, Dubey JP, Labelle P, Martineau D. 2004. Seroprevalence of *Toxoplasma gondii* in Canadian pinnipeds. Journal of Wildlife Diseases 40(2):294-300.

Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, Dodd E, Harris MD, Ames JA, Packham AE, Conrad PA. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). International Journal for Parasitology 32(8):997-1006.

Oksanen A, Tryland M, Johnsen K, Dubey JP. 1999. Serosurvey of *Toxoplasma gondii* in North Atlantic marine mammals by the use of agglutination test employing whole tachyzoites and dithiothreitol. Comparative Immunology, Microbiology and Infectious Diseases 21(2):107-114.

Oksanen A, Asbakk K, Prestrud KW, Aars J, Derocher AE, Tryland M, Wiig Ø, Dubey JP, Sonne C, Dietz R, Andersen M, Born EW. 2009. Prevalence of antibodies against *Toxoplasma gondii* in polar bears (*Ursus maritimus*) from Svalbard and east Greenland. Journal of Parasitology 95(1):89-94.

Philippa JD, Leighton FA, Daoust PY, Nielsen O, Pagliarulo M, Schwantje H, Shury T, Van Herwijnen R, Martina BEE, Kuiken T, Van de Bildt MWG, Osterhaus ADME. 2004. Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. The Veterinary Record 155(5):135-140.

Prestrud KW, Asbakk K, Fuglei E, Mørk T, Stien A, Ropstad E, Tryland M, Gabrielsen GW, Lydersen C, Kovacs KM, Loonen MJJE, Sagerup K, Oksanen A. 2007. Serosurvey for *Toxoplasma gondii* in arctic foxes and possible source species of infection in the high Arctic of Svalbard. Veterinary Parasitology 150(1):6–12.

R-Development Core Team. 2006. R: A Language and Environment for Statistical Computing, Vienna: R Foundation for Statistical Computing, ISBN 3-900051-07-0. Available: http://www.R-project.org.

Rah H, Chomel BB, Follmann EH, Kasten RW, Hew CH, Farver TB, Garner GW, Amstrup SC. 2005. Serosurvey of selected zoonotic agents in polar bears (*Ursus maritimus*). The Veterinary Record 156(1):7-13.

Ripert C. 2000. Reactive hypereosinophilia in parasitic diseases. La Revue du Practicien 50(6):602-607.

Rodgers SJ, Baldwin CA. 1990. A serologic survey of Oklahoma cats for antibodies to feline immunodeficiency virus, coronavirus, and *Toxoplasma gondii* and for antigen to feline leukemia virus. Journal of Veterinary Diagnostic Investigation 2(3):180-183.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds AJ. 2003. Fingerprints of global warning on wild animals and plants. Nature 421(6918):57-60.

Ross PS. 2002. The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. Human and Ecological Risk Assessment 8(2):277-292.

Saliki JT, Cooper EJ, Gustavson JP. 2002. Emerging morbilliviral infections of marine mammals-development of two diagnostic approaches. Annals of the New York Academy of Sciences 969(1):51-59.

Tryland M. 2000. Zoonoses of arctic marine mammals. The Infectious Disease Review 2(2):55-64.

Tryland M, Neuvonen E, Huovilainen A, Tapiovaara H, Osterhaus ADME, Wiig Ø, Derocher AE. 2005. Serologic survey for selected virus infections in polar bears at Svalbard. Journal of Wildlife Diseases 41(2):310–316.

Zarnke RL, Dubey JP, Kwok OCH, Ver Hoef JM. 1997. Serologic survey for *Toxoplasma gondii* in grizzly bears from Alaska. Journal of Wildlife Diseases 33(2):267–270.

Zarnke RL, Dubey JP, Kwok OCH, Ver Hoef JM. 2000. Serologic survey for *Toxoplasma gondii* in selected wildlife species from Alaska. Journal of Wildlife Diseases 36(2):219–224.

Zarnke R, Dubey JP, Ver Hoer J, McNay ME, Kwok OCH. 2001. Serologic survey for *Toxoplasma gondii* in lynx from interior Alaska. Journal of Wildlife Diseases 37(1):36-38.

Zarnke RL, Ver Hoef JM; DeLong RA. 2004. Serologic survey for selected disease agents in wolves (*Canis lupus*) from Alaska and the Yukon Territory, 1984–2000. Journal of Wildlife Diseases 40(4):632-638.

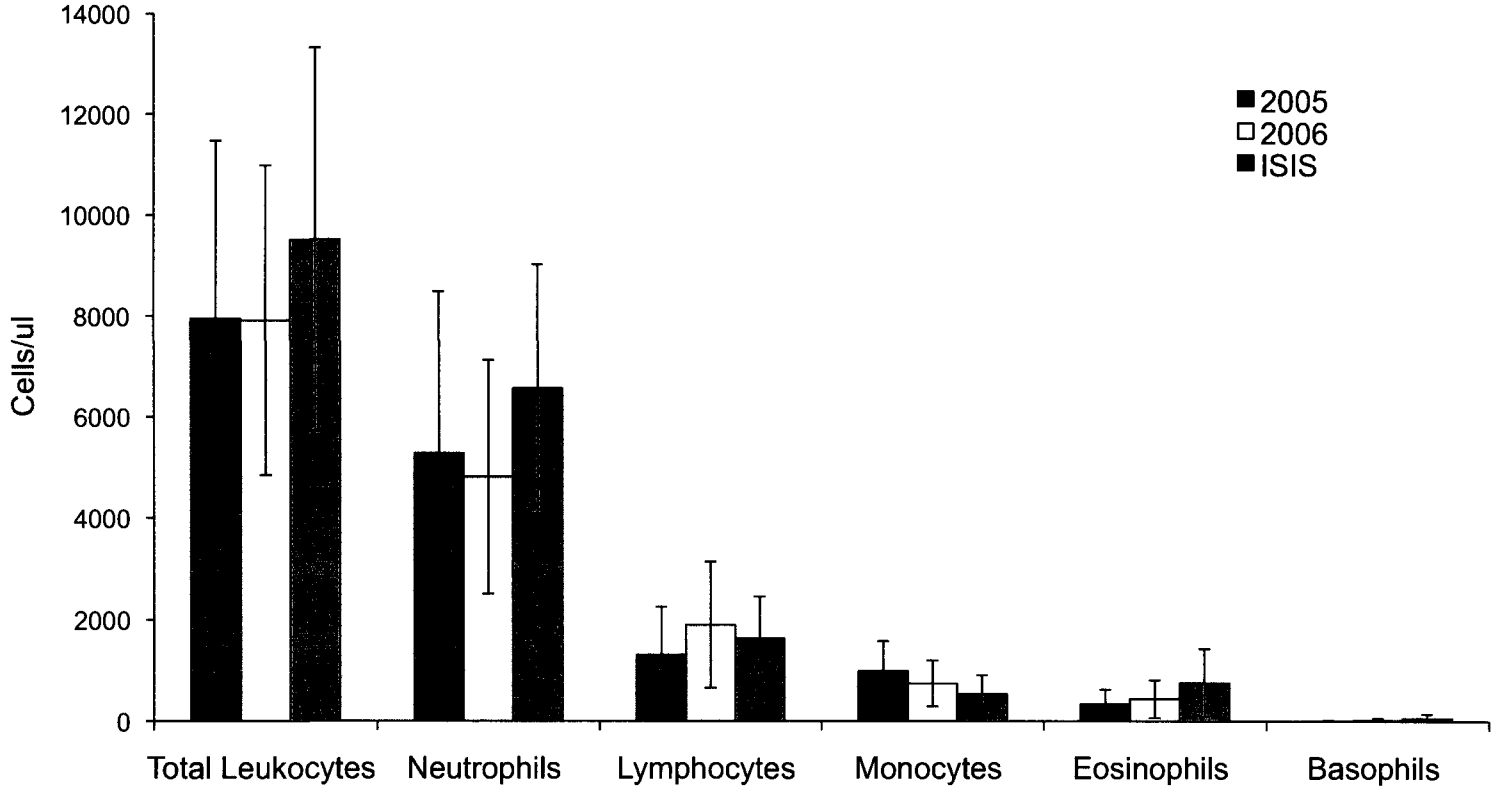

Figure 2.1 Statistics for blood cell type counts for southern Beaufort Sea, Alaska polar bears for 2005, 2006, and for captive polar bears in the International Species Information System (ISIS) database for captive polar bears.

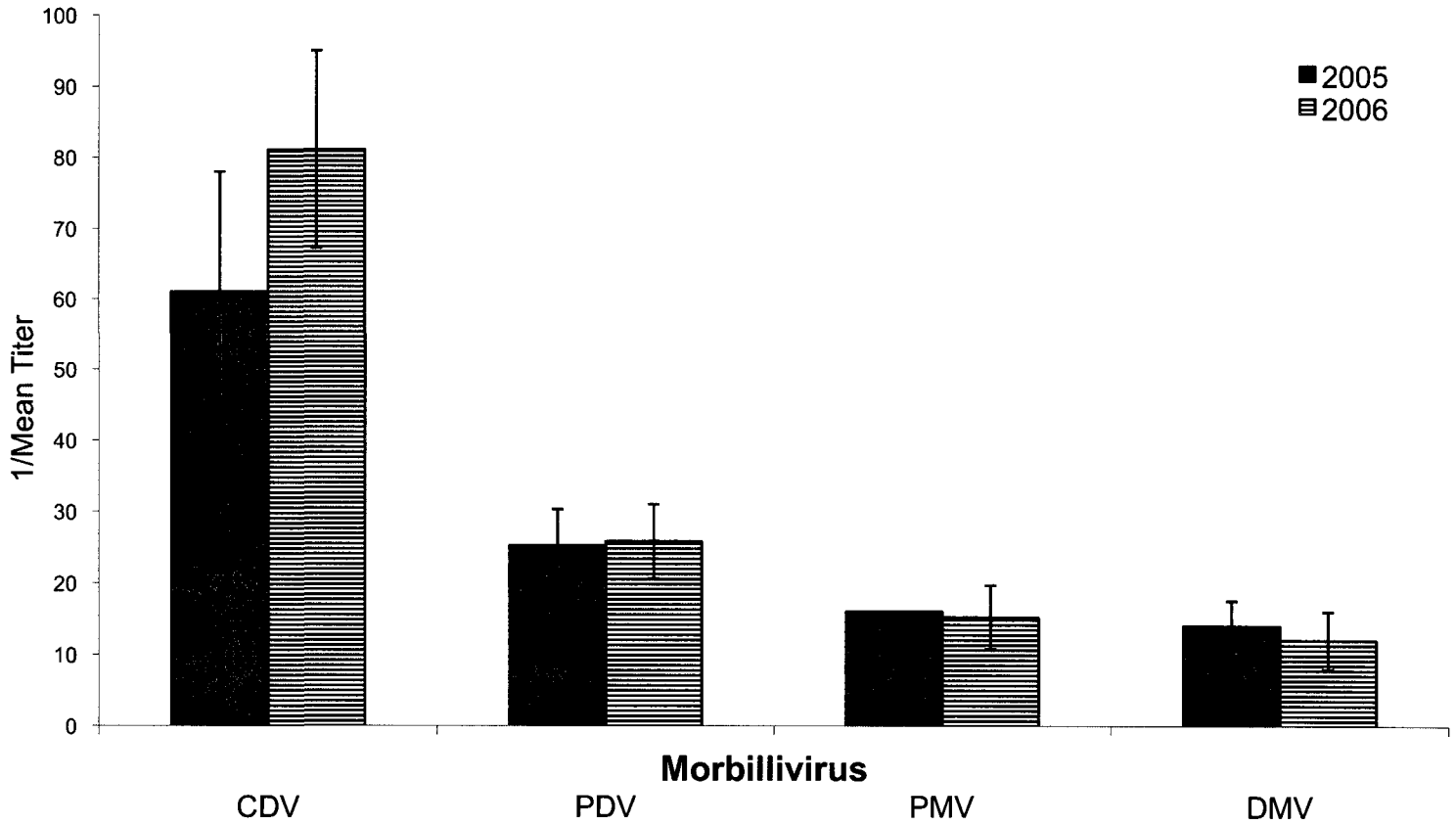

Figure 2.2 Mean titers for southern Beaufort Sea, Alaska polar bears seropositive in morbillivirus serum neutralization differential (1/ mean ± SE).

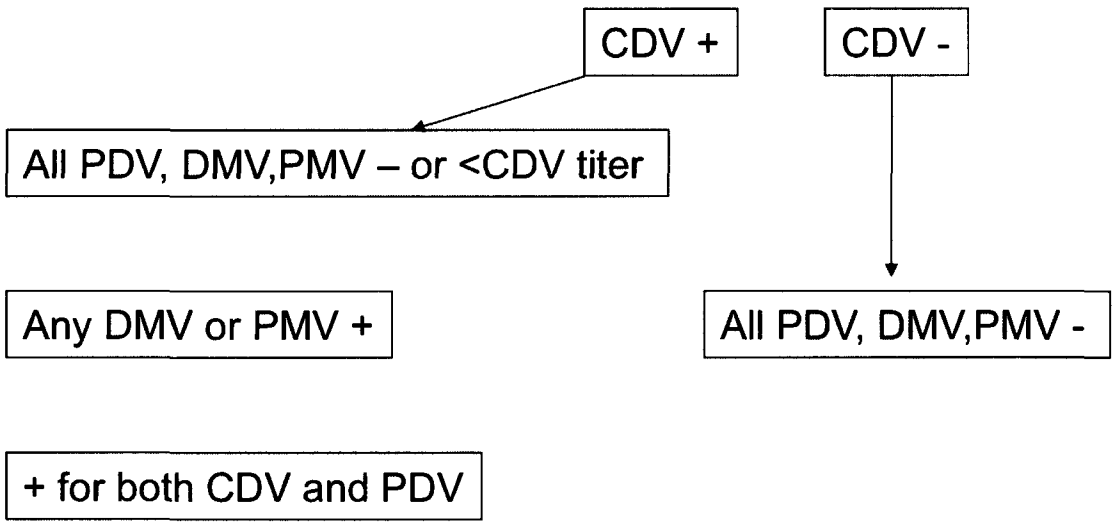

Figure 2.3 Conceptual model for morbillivirus serum neutralization assay cross-reactivity for southern Beaufort Sea, Alaska polar bears.

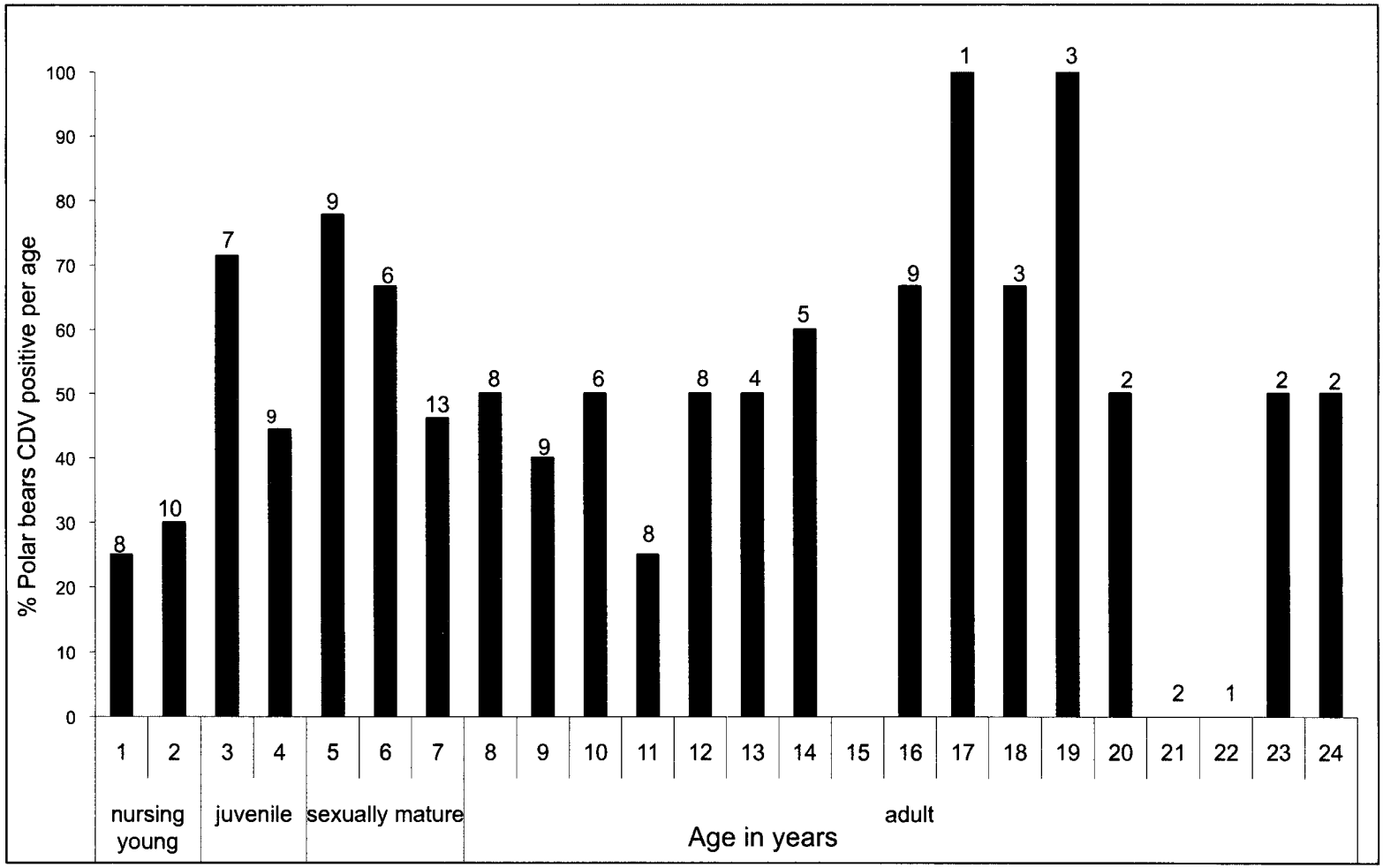

Figure 2.4 Percentage of southern Beaufort Sea, Alaska polar bears CDV seropositive by age (years 2005 and 2006 combined). Numbers above bars represent sample size for each age category.

Table 2.1 Antibody prevalence rates (number positive) for polar bears captured in 2005, 2006, and both years (total) as determined using serum virus neutralization assay. By: (a) pathogen (b) gender and (c) age class including odds ratios.

(a)

Pathogen	Total	2005 (63)	2006 (73)
CDV	50.0%	49.2% (31)	50.7% (37)
PDV	24.3%	20.6% (13)	27.4% (20)
DMV	4.4%	6.3% (4)	2.7% (2)
PMV	4.4%	1.6% (1)	6.8% (5)
Toxoplasma gondii	13.2%	12.7% (8)	13.7% (10)

canine distemper (CDV), phocine distemper (PDV), porpoise morbillivirus (PMV) and dolphin morbillivirus (DMV).

(b)

		Sample year (N)	
Cohort	**Total**	**2005 (63)**	**2006 (67)**
Sex class	**Prevalence**	**Prevalence**	**Prevalence**
Females	50.7%	61.8% (21)	40.5% (15)
Males	47.5%	34.5% (10)	60.0% (18)

(c)

			Sample year (N)	
Cohort	**Total**		**2005 (62)**	**2006 (68)**
Age class (years)	**Prevalence**	**OR (95% CI)α**	**Prevalence β**	**Prevalence β**
1-2	20.0%	1	14.3% (1)	23.1% (13)
3-4	25.0%	1.32 (0.16-9.90)	28.6% (2)	20.0% (5)
5-7	78.6%	13.2 (2.19-112.83)	100.0% (5)	66.7% (9)
≥ 8	56.0%	5.01 (1.45-22.33)	53.5% (23)	58.5% (41)

[α] $\chi 2 = 15.96$, df = 3, p = 0.001; for odd ratio calculations comparing all age classes to 1-2 years.

[β] Cochran-Mantel-Haenszel $\chi 2 = 16.244$, df = 4, p = 0.003; for homogeneity in prevalence among age classes stratified by year.

Table 2.2 Correlations between hematological and serological parameters for southern Beaufort Sea, Alaska polar bears.

		Sample year (N)							
		2005 (61)			2006 (67)		2005 + 2006 (128)		
Variable	**By Variable**	**r**	**p**	**MD**	**r**	**p**	**r**	**p**	**MD**
CDV titer	Total Leukocytes	-0.36	0	-26.10%	-0.22	0.08	-0.28	0	-22.60%
CDV titer	Neutrophils	-0.27	0.03	-28.20%	-0.11	0.36	-0.19	0.03	-23.90%
CDV titer	Eosinophils	-0.31	0.01	-50.40%	-0.11	0.41	-0.18	0.04	-29.90%
CDV titer	Monocytes	-0.27	0.03	-33.10%	-0.16	0.22	-0.22	0.01	-27.80%

Variables were log transformed before correlations calculated. r = Pearson product-moment correlation coefficient, p = P-value where $\alpha < 0.05$, and MD = % change in count means.

CHAPTER 3

Arctic Fox (*Vulpes lagopus*) Morbillivirus Ecology in Northern Alaska: Characterization of phosphoprotein gene fragment and comparison to serology of polar bears (*Ursus maritimus*)[1]

3.1 ABSTRACT

Morbilliviruses pass between terrestrial and aquatic environments but their presence has not yet been confirmed in arctic foxes, which live at the terrestrial-marine interface and may therefore serve as an ecological link in morbillivirus transmission. Because increasing contaminant burdens and changing climate are likely to affect morbillivirus transmission dynamics in the Arctic, an understanding of the current ecology of these viruses is necessary to assess future variation. We examine potential morbillivirus transmission pathways among Arctic carnivores of northern Alaska using serology and molecular epidemiologic techniques. We characterize a fragment of the polymerase co-factor phosphoprotein (P) gene of morbillivirus RNA isolated from arctic fox (*Vulpes lagopus*) and compare antibody cross reactivity profiles in serum neutralization assays for morbillivirus for arctic fox and polar bears (*Ursus maritimus*) captured in the Prudhoe Bay area over a three years period. Three of 11 arctic fox tested were positive for

[1] Prepared for submission in the format of EcoHealth: Kirk CM, Beckmen K, Burek K Follmann E, O'Hara TM. 2010. Arctic Fox (*Vulpes lagopus*) Morbillivirus Ecology in Northern Alaska: Characterization of phosphoprotein gene fragment and comparison to serology of polar bears (*Ursus maritimus*).

morbillivirus with identical sequences. Phylogenetic analysis using maximum likelihood clustered the arctic fox strain among "Arctic-like" isolates of canine distemper virus (CDV). Morbillivirus variants clustered by geographic rather than host origin as is typically observed for this virus. Antibodies reacting with phocine distemper virus (PDV) antibodies were detected in 90.1% (10/11), dolphin morbillivirus (DMV) in 72.7% (8/11), CDV in 54.6% (6/11), and porpoise morbillivirus (PMV) in 36.4% (4/11) of foxes using serum neutralization assays. These results contrast with those found for polar bears where prevalences were 24.3%, 4.4%, 50%, and 4.4%, respectively. Although morbillivirus in arctic fox appears "canine" at the genetic (phosphoprotein) level, it cannot be distinguished from marine varieties using virus serum neutralization assays, especially for animals with recent exposure. Serology suggests morbillivirus circulating among polar bears is distinct from that which infected sympatric arctic foxes in 2007 or that polar bears have a different serologic response to the same virus.

3.2 INTRODUCTION

Distemper is a complex disease affecting enteric, respiratory, lymphoid and neural systems (Appel, 1987). The causative agent is a single stranded negative sense RNA virus in the genus *Morbillivirus* that is transmitted primarily via aerosolization of mucosal exudate, for example through sneezing or scavenging (Alexander and Appel, 1994; Cleaveland et al., 2000). Canine distemper virus (CDV) was historically known to infect only members of the families Canidae (dogs, wolves, foxes), Procyonidae (raccoon, coati mundi), and Mustelidae (ferret, mink, skunk). More recently it has been reported in all families of terrestrial carnivores and some marine mammals (Deem et al., 2000).

Morbillivirus epizootics in wildlife can be associated with anthropogenic factors such as pathogen spillover, contaminant-related immunosuppression, habitat modification, or combinations thereof. However, the development of naïve and thus vulnerable subpopulations lacking specific immunity to morbilliviruses also may be a mechanism for periodic epizootics as a critical number (density) of vulnerable hosts is reached. Interactions among age, stress, nutrition, population dynamics, and climate can affect wildlife health and therefore susceptibility to infection (Ross, 2002). In outbreaks among marine mammals contaminant-related immunosuppression and host range shifts due to warming waters and overfishing are hypothesized to have facilitated outbreaks in Northern Europe. This may have occurred as naïve seal species (e.g. harbor seals) moved north, coming into contact with morbillivirus reservoir species of Arctic pinnipeds

(Hammond et al., 2002; Harkonen et al., 2002). As a result of such phenomena, the Arctic has been a suspected morbillivirus source of some large-scale marine mammal mortality events in the past (e.g., Duignan et al., 1997; Barrett et al., 1995).

In the last 20 years, four morbilliviruses have emerged as significant causes of morbidity and mortality in marine mammals: CDV, PDV, dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV). Rima et al. (1995) suggested that DMV and PMV are different strains of the same species (CMV); however, van de Bildt et al. (2005) found that the viruses are more divergent than some distantly related measles viruses and thus not likely the same species. These four morbilliviruses are very similar genetically and antigenically and cross reactivity is known to occur in serological assays (Saliki et al., 2002). Morbilliviruses apparently pass between terrestrial and aquatic environments. Canine distemper virus from terrestrial species was implicated for example in outbreaks among Baikal seals (*Phoca siberica*; Mamaev et al., 1995) and Caspian seals (*Phoca caspica*), and was thought to be transmitted to seals from domestic dogs (Kuiken et al., 2006). Conversely, PDV was suggested to be the cause of an outbreak of distemper among farmed mink (*Mustela vison*) near the coast in Denmark (Blixenkrone-Møller et al., 1989).

Arctic fox (*Alopex lagopus*) are found throughout the circumpolar arctic and like polar bears (*Ursus maritimius*) exploit both terrestrial and marine environments for food (Pamperin et al., 2008). The foraging ecology of these species makes them good sentinels

for monitoring the introduction of infectious agents into the arctic food web and also the movement of infectious agents between the terrestrial and marine environments. Polar bears seropositive for CDV-like viruses have been found across the Arctic including Canada and Svalbard, where prevalence has been documented at 24% and 8%, respectively (Cattet et al., 2004; Tryland et al., 2005), and in Alaska and Russia at 26-46% (Follmann et al., 1996). However, there are no published records of morbillivirus in arctic foxes. In 1994, an assessment of 99 Alaska arctic fox (Prudhoe Bay area) detected no evidence of morbillivirus in serum or neural tissue (Ballard et al., 2001). Early hypotheses regarding the exposure of polar bears to morbillivirus implicated the consumption of PDV-infected seal prey. Serological studies examining the reactivity of polar bears serum to all 4 morbilliviruses, however, demonstrated a greater reactivity to CDV antigen (virus) than to PDV, DMV or PMV (CDV, PDV, DMV, and PMV, Garner et al., 2000; CDV and PDV, Cattet et al., 2004). Given the relatively high seroprevalence of polar bears to CDV-like antigen and the apparent lack of clinical manifestations, the virus may be able to replicate within polar bears without adverse effects. Thus they may serve as a reservoir for other sympatric carnivores, such as the arctic fox, pinnipeds, and domestic dog.

Canine distemper-like antibodies have also been detected in terrestrial arctic-dwelling carnivores in Alaska including brown bears (Chomel et al., 1998; Philippa et al., 2004), and wolves (*Canis lupus*) (Zarnke et al., 2004). Serologic evidence of morbillivirus has not been documented in marine mammals of the Alaskan Arctic other than polar bears;

however, antibodies to PDV have been reported in various pinniped and cetacean species in the Canadian Arctic including ringed seals (*Pusa hispida*), the polar bear's principle prey (Duignan et al., 1997). There is evidence that morbillivirus is endemic in the North Atlantic and Barents Sea harp seal (*Phoca groenlandica*) populations where PDV seroprevalence ranging from 67% to 100% (N=183) have been reported (Tryland et al., 2005). Recently, Goldstein et al. (2009) identified PDV via genetic analysis in Northern sea otters (*Enhydra lutis*) in Alaska, the first documentation of morbillivirus in any Pacific species. These authors hypothesize that sea ice reduction may have altered seal haul-out and migration patterns, resulting in contact between Atlantic, Arctic, and Pacific Ocean species. These findings have generated concern of infectious disease threats to several sympatric and declining Pacific marine mammals.

Climate change and anthropogenic influences (e.g. industry, proximity to growing communities and/or food sources) may be altering ecology of disease agents and host species in the Arctic. It is therefore essential to establish baselines of prevalence and current transmission dynamics of infectious agents so that change over time may be assessed. The impetus of this study was to elucidate morbillivirus transmission pathways among Alaskan arctic carnivores, using molecular epidemiologic techniques including sequence analysis and phylogeny as well as serology. To date, morbillivirus in arctic fox has not been described. We provide baseline information on morbillivirus seroprevalence in arctic fox. We also characterize a fragment of the polymerase co-factor phosphoprotein (P) gene of morbillivirus RNA isolated from arctic fox, performed phylogenetic analyses

and compared cross reactivity profiles in serum neutralization assays for morbillivirus for arctic fox and polar bears. We focus on two species included in the conceptual model for morbillivirus transmission for this region (Figure 3.1). This model can be generalized for pathways of related infectious agents (similar transmission characteristics).

3.3 METHODS

3.3.1 Arctic Fox Epizootics and Pathology Investigation

During January through May 2007, a rabies and morbillivirus mortality event occurred on the North Slope of Alaska among arctic and red fox (*Vulpes vulpes*). Foxes were submitted to Alaska Department of Fish & Game (ADF&G) by oil industry personnel when found dead or when suspected to be infected with rabies on the basis of behavior including, aggression and lack of fear of humans. Personnel from ADF&G and University of Alaska Fairbanks (UAF) performed complete necropsies. Fox brains were submitted to the Alaska State Virology Laboratory (ASVL) for rabies testing. Tissues for histopathology were prepared at Veterinary Services, UAF. Immunohistochemistry was performed at Washington Animal Disease Diagnostic Laboratory (WADDL) and histologic evaluations for morbillivirus were performed at the Alaska Veterinary Pathology Services (AVPS, K. Burek).

3.3.2 Animal Capture and Sampling

We used Tomahawk Live Traps© baited with tuna in oil to capture arctic fox throughout the Prudhoe Bay area approximately between 148°60' and 147°50' longitude and from

the coast to approximately 70°00 latitude between June 30th and July 11th, 2007 (Figure 3.2). Eleven arctic fox (5 males, 6 females) were captured and transported to a holding facility. Animals were transferred to squeeze cages and injected (intramuscularly) with anesthetic agents by syringe [~ 6 mg/kg ketamine (Ketaset®; Fort Dodge, IA)/ 3 mg/kg xylazine (Boehringer Ingelheim Vetmedica Inc.; St. Joseph, MO)] in the hind leg. Blood was collected from the jugular vein (Vacutainer, BD Biosciences; Franklin Lanes, NJ) into evacuated blood collection tubes for whole blood and serum; and PAXgene Blood RNA tubes (Qiagen; Valencia, California) for RNA isolation. Sex, age class, physical condition, body mass and morphometrics were recorded. Age class was designated as juvenile (< 1 year) or adult (> 1 year) based on tooth wear. Viral CULTURETTE™ Collection and Transport Systems (BD Biosciences; Franklin Lanes, NJ) were also used to swab nasal cavities for RNA extraction. Animals were given a passive integrated transponder (PIT) tag subcutaneously to enable identification of animals in future contacts. Animals were released at site of capture following observation and recovery. This project was approved by the UAF IACUC (Assurance 07-30). Polar bears were captured in the Springs of 2005 and 2006 (late March through early May) out of logistic bases in Barrow, Prudhoe Bay, and Kaktovik. Blood was collected from the femoral vein or artery (Vacutainer, BD Biosciences; Franklin Lanes, NJ) into evacuated blood collection tubes for whole blood and serum and PAXgene Blood RNA tubes (Qiagen, Valencia, California) for RNA isolation. Viral CULTURETTE™ Collection and Transport Systems (BD Biosciences; Franklin Lanes, NJ) were also used to swab nasal

cavities for morbilliviral RNA detection. Sampling was conducted as described in Kirk et al. (2010).

3.3.3 Serology

Sera derived from blood collected without anticoagulant were separated by centrifugation at 1,500 x g for 5 minutes (TRIAC, Clay Adams Co., Parisippany, NJ, USA) and frozen at -20°C. Sera were stored at -70°C upon return from the field for later serological assays. Polar bear and arctic fox serum was assayed for morbilliviral antibody at the Oklahoma State University Animal Disease Diagnostic Laboratory (OADDL, Stillwater, OK USA) via differential serum neutralization (SN) assay for four morbilliviruses (CDV, DMV, PDV, PMV) as described by Garner et al. (2000). Arctic fox serum samples were also assayed for morbilliviral antibodies at Athens Diagnostic Laboratory, College of Veterinary Medicine at the University of Georgia (UGA) via differential serum neutralization (SN) assay for three morbilliviruses (CDV, PDV, CMV). Polar bear serum was screened at only one laboratory since the intent of testing was surveillance as part of the health assessment study, while arctic fox were tested in response to a suspected morbillivirus-based mortality event.

Results from both laboratories are expressed as the reciprocal of the highest dilution that completely neutralized 100% of the respective challenging virus using a cut-off of 1:8 for determining presence of specific antibodies. Seroprevalence and 95% confidence intervals for mean prevalences were calculated for polar bears in 2005, 2006, and for

arctic foxes in 2007 as described in Hughes-Hanks et al. (2005). Results for arctic fox serology were compared between laboratories. In order to compare morbilliviral exposure between arctic fox and polar bear, we compared cross reactivity profiles for the serum neutralization differential assay for data obtained from OADDL. Since the data were not normally distributed the non-parametric Wilcoxon signed-rank test was used to compare reciprocal mean titers for polar bears and arctic fox for each virus tested at OADDL.

3.3.4 Molecular Analyses

RNA was extracted from fox carcasses obtained from the North Slope from retropharyngeal lymph nodes, brain and/or lung depending upon sample quality and availability. Tissues were either preserved in RNA*later*® (Applied Biosystems; Foster City, California) or frozen and were processed using the FastPrep® Instrument with the FastRNA® Pro Green Kit according to the manufacturer's instructions (MP Biomedicals; Solon, Ohio). RNA was extracted from blood using the PAXgene Blood RNA kit (Qiagen; Valencia, California) according to the manufacturer's instructions. RNA was extracted from nasal swab samples using TRI REAGENT® LS [Manufacturer's protocol (1995), Molecular Research Center, Inc., Cincinnati, OH]. Sample integrity was assessed via amplification of a housekeeping gene, β-actin (Kraft et al., 1995). For morbillivirus, we used the universal primer set, which amplifies a well-conserved region of the polymerase cofactor, phosphoprotein, for all known morbilliviruses (Barrett et al., 1993). Reverse transcription reactions were performed using SuperScript™ III One-Step RT-PCR System with Platinum® Taq, according to the manufacturer's instructions (Invitrogen,

Carlsbad, California). Amplified products of expected molecular masses were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, California) and then cloned using TOPO TA Cloning® kit according to the manufacturer's instructions (Invitrogen, Carlsbad, California). Sequencing was performed in duplicate in both the forward and reverse directions using vector specific primers at the Core Facility for Nucleic Acid Analysis Institute of Arctic Biology, UAF with use of the BigDye™ Terminator Kit (Applied Biosystems, Foster City, California) and an automated sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems) according to the manufacturer's instructions. Primer sequences were removed prior to bioinformatics analyses. The complete sequence assemblies were created using Lasergene® v7.0 (DNASTAR, Inc., Madison, Wisconsin) using nucleotide data with quality higher than 20. Sequences resulting from a BLAST search of the National Center for Biotechnology Information (NCBI) database were imported and aligned with fox sequences using Clustal W in the Lasergene® v7.0 software package. The reference CDV nucleotide sequences used for phylogenetic analyses were obtained from the NCBI database with assigned GenBank accession numbers given below. These sequences were chosen based on BLAST results (e.g. those with homology to the arctic fox strain) and geographic origin such that at least 2 isolates from each region of both canine and non-canine origin where available were represented in the analysis. Only one of the 2 Missouri dog isolates (18133) with high homology to arctic fox virus were included in the analysis as sequences were identical. Appropriate likelihood models were determined using the Akaike Information Criterion implemented in ModelTest 3.7, PAUP* 4.0b10 (Posada and Crandall, 1998; Posada and Buckley,

2004). PHYML (Guindon and Gascuel, 2003) was used to construct a maximum likelihood (ML) tree for partial P gene sequences using the GTR model for nucleotide substitution with transition/transversion ratio, proportion of invariable sites, and gamma distribution empirically determined with 6 nucleotide substitution categories, 1000 bootstrap replicates and the NNI+SPR tree topology search operation for Arctic Fox Alaska USA, 2007; Siberian Seal Russia, 1988 (AF259551); Alaska Sled Dog, 1996-1997 (Maes et al., 2003); Missouri Dog case 18133 USA, 2004 (AY964107) ; Raccoon USA, 2003 (AY321298); Lynx Canada 2008 (FJ240229); Dog A75/17 USA, 1975 (AF164967); Dog Hamamatsu Japan, 1992 -1994 (AB028915); Dog Jujo Japan (AB028916); Dog Yanaka Japan, 1992 -1994 (AB028914); Ferret Germany, 1989 (AF259550); Dog 5804 Germany, 1990 (AY386315); Dog Germany, 1993 (AF259549); PDV-1 (X75960); DMV (AJ608288); CMV (AF333347); Dog Kazhakstan, 2007 (EU888880); Caspian Seal Kazhakstan, 2007 (EU594261), CDV Rockborn vaccine (AF1814460); and CDV Onderstepoort vaccine (AF305419).

3.4 RESULTS

3.4.1 Serology

Interlaboratory differences between OADDL and UGA were found in cross reactivity profiles for individual arctic fox, however results were consistent in that the same single individual did not test positive for any virus in either differential panel. Based on UGA data, seroprevalences (Table 3.1a) and titer ranges for the three viruses were similar [CDV (titer range = 1:16-1:256), PDV (titer range = 1:16-1:128), CMV titer range =

1:16-1:128)]. Based on OADDL data however, 91% (N=11) of arctic fox demonstrated prevalence (Table 3.1a) to PDV (titer range = 1:8-1:128) with mean titers varied and ranges roughly equal [(CDV titer range = 1:8-1:128), (DMV titer range = 1:12-1:96), (PMV titer range = 1:16-1:128)] (Figure 3.3). The number of arctic fox seropositive for each virus tested via SN assay by both OADDL and UGA is provided in Table 3.1b.

Polar bear seroprevalence for CDV in both 2005 and 2006 was relatively high and antibodies to PDV, DMV and PMV were also detected (Table 3.1a). Mean titers for seropositive polar bears were highest for CDV (titer range = 1:8-1:512), followed by PDV (titer range = 1:8-1:96), DMV (titer range = 1:8-1:24) and lowest for PMV (titer range = 1:16-1:32) as indicated in Figure 3.3. All animals seropositive to DMV, PDV, and/or PMV had a higher titer to CDV. Of all polar bears seropositive for CDV, 48.5% were also seropositive for PDV. Any animal seropositive for DMV or PMV were also seropositive for both CDV and PDV. Reciprocal mean titers were significantly greater for polar bears sampled in 2005 than in 2006 and as compared to arctic fox (Figure 3.3). No other significant differences in mean titers were found.

3.4.2 Molecular Analyses

During the mortality event, 19 foxes (12 arctic and 7 red) were subject to complete necropsy and histopathological examinations. Of these 19 animals, 11 were tested for morbillivirus by RT-PCR and 27% (3/11) were positive. All 3 were arctic fox. Six of the 19 animals evaluated in the mortality event were positive for CDV antigen via

immunohistochemistry (IHC), including one red fox that was not tested via RT-PCR. Four of these IHC positive animals were not tested via RT-PCR due to sample availability, one was negative via RT-PCR, and one was positive. For the animal where assay results for morbillivirus did not agree, different tissues were tested. Lung was tested via IHC whereas only the retropharyngeal lymph node was available for RT-PCR. Of foxes testing positive for CDV in either assay, two were adults (1 male and 1 female), three were juveniles (1 male and 2 females) and the age of two could not be determined due to damage to the carcass resulting from vehicular collision and scavenging. One of three foxes that tested morbillivirus positive via RT-PCR presented clinical signs and pathologic lesions consistent with canine distemper viral infection and also tested positive via IHC (K. Burek; data not presented). One co-infection of rabies and CDV in a red fox was diagnosed. Viral RNA was not amplified from nasal swabs of live-captured arctic fox or polar bears. The amplified P gene fragments were identical for the three positive arctic fox cases. Alignment with sequences available from GenBank revealed that the arctic fox isolate differed from a strain derived from two North American dogs (Missouri, June through October of 2004) by only 1 nucleotide transition (G to A) (Figure 3.4). The arctic fox sequence also displayed very high homology with a sequence derived from a Siberian seal during a CDV outbreak in Lake Baikal, Russia (Mamaev et al., 1995) and with a sequence obtained from an Alaskan dog during a CDV outbreak among sled dogs in Kotzebue (Maes et al., 2003). The arctic fox isolate differed by only 2 nucleotides (both transitions and transversions) from each of these sequences (Figure 3.4). Phylogenetic analysis of the 389 bp P gene sequence using maximum likelihood

clustered the arctic fox isolate among these sequences, however it did not form a clade with any sequences used in the analysis (Figure 3.5).

3.5 DISCUSSION

3.5.1 Serology Comparisons

Comparison of morbillivirus serological cross reactivity profiles shows a markedly different pattern for polar bear and arctic fox suggesting viruses to which they have been exposed are distinct and/or the antibody responses between these ursids and canids are dissimilar. Without viral sequences available from polar bears to compare, we cannot rule out differences due to variation in host immune response. Results for polar bears were consistent with those found by other researchers who concluded that the morbilliviral antigen to which Alaska polar bears had been exposed was most likely of terrestrial origin because the greatest number of animals demonstrated antibodies to CDV and each animal presenting a titer for any of the other 3 morbilliviruses tested (PDV, DMV, and PMV) presented a higher titer to CDV (Garner et al., 2000; this study). Based on OADDL data, arctic fox in contrast demonstrated the greatest prevalence to PDV, and titer levels for seropositive animals were quite varied and did not show the consistent pattern we observe in polar bears. Arctic fox serology results from UGA were not identical to those from OADDL in titer level for each virus, however results were the same regarding which animals were seropositive for at least one of the viruses tested in each panel (Table 3.1b). Based on OADDL data, most animals (N=10) appeared to seroconvert to PDV+ while based on UGA data 9 animals seroconverted to PDV+ and

CMV+ while 10 seroconverted to CDV+. The UGA and OADDL data together do not provide clear support for either marine or terrestrial origin. It is also possible that antibodies observed in arctic fox sampled after the epizootic did not result from exposure to the same virus, which caused mortality 3 months prior to sampling of live captured foxes. Foxes sampled in the summer could have seroconverted as a result of an infection occurring prior to the mortality event from which we obtained viral RNA. These data represent the first published serological profile for morbillivirus in arctic fox therefore there is no other data available for comparison. The results of this study underscore the limitations of using serology to elucidate transmission pathways of morbillivirus in wildlife and for specific identification of the antibody-inciting agent.

3.5.2 Phylogenetics

Of seven confirmed CDV cases examined during an outbreak among breeder dogs in Missouri there were two strains demonstrating high homology with the arctic fox strain examined in this study. These variants were determined by Pardo et al. (2005) to be genetically distinct from viruses previously detected within the continental United States and most closely related to a Siberian seal isolate. The infected dogs originated from a breeder within the state and had no history of recent travel, however, neither travel history for the bitch and stud (or other animals at the facility), nor breed information were provided in the manuscript. The authors suggested that the virus may have originated from non-canine species or may have been transmitted from dogs to other species. The arctic fox sequence demonstrated the second greatest percentage homology with a strain

of CDV previously documented in Alaska among sled dogs and a high level of homology had previously been demonstrated between this Missouri dog isolate and the Alaska sled dog isolate (Pardo et al., 2005). A later study performed by McCarthy et al. (2007) to assess the role of selection and recombination in shaping viral genetic diversity and driving the emergence of CDV in non-dog hosts included the Missouri dog isolates in the "Arctic-like" cluster along with the Alaskan sled dog and Siberian seal. In phylogenetic analyses of CDV, isolates generally cluster by geographic region rather than host origin as is demonstrated here (Harder and Osterhaus, 1997). In the maximum likelihood analysis isolates from a dog and seal in Kazakhstan formed a clade, as did isolates from Japanese dogs and German dogs (Figure 3.5). The Siberian seal and Alaska sled dog, as well as 2 North American isolates [lynx (*Lynx canadensis*) and USA dog] also formed clades (Figure 3.5). Furthermore, the arctic fox isolate grouped in the "Arctic-like" cluster along with the Siberian seal, Alaska sled dog, and the Missouri dog case. The exception here is the German ferret isolate, which did not cluster among European strains but appears to be more closely related to the "Arctic-like" strains. This pattern whereby isolates cluster by geographic rather than host species or genus origin in phylogenetic analysis achieves greater resolution and statistical support (e.g. bootstrap values) when the haemagglutinin (H) and fusion (F) genes are examined because there tends to be more sequence divergence in these genes among morbilliviruses. The H and F proteins are required for virus attachment and entry and therefore these genes are under greater selection pressure, which is hypothesized to result in the greater sequence variability observed across morbilliviral species.

The power of our phylogenetic assessment was somewhat limited due to the high conservation and therefore limited diversity of the P gene and because fewer sequences are available in the public databases as compared to other morbilliviral genes. We targeted this gene in RT-PCR precisely because it is highly conserved and therefore lent the greatest possibility of success in amplifying viral nucleic acid from our samples. For this initial study, we chose to target the only gene for which there exists a "universal primer set" capable of amplifying all morbilliviruses as the arctic fox morbillivirus was "novel" and results of serology did not indicate that canine specific primers would be useful. Examining the maximum likelihood tree, we can however clearly see that the arctic fox strain is more closely related to CDV than PDV, DMV, or CMV and appears to cluster among the "Arctic-like" isolates. Furthermore, the tree supports the hypothesis that the arctic fox isolate is distinct from vaccine strains and typical North American, European, Eurasian and Asian field strains based on sequences available in Genbank.

3.5.3 Other Distemper Epizootics in the Arctic

During the same time period that cases were reported in Alaska for arctic fox an outbreak of CDV occurred among sled dogs in the Canadian Arctic throughout Nunavut and the Northwest Territories (Campbell et al., 2007). Samples were confirmed positive via IHC; however viral sequences were not examined. Because the epizootic occurred in widely separated locations, arctic fox were suggested to be the source of infection. There were however, no reported cases of CDV in foxes in these regions during the epizootic. Although no cases of CDV were reported for Alaska dogs during this time, the number of

dogs present in Alaska arctic communities has drastically decreased since the introduction of the snow machine as an alternative means for transportation (Rausch, 2003). In addition, many dogs living in Alaskan villages are now vaccinated through government sponsored immunization programs (http://www.uscgalaska.com/go/doc/780/310669/) and via private veterinarians.

Epizootics of CDV have occurred previously in the Arctic. In the winter of 1987-1988 an outbreak occurred over a broad geographic range in the Canadian Arctic concurrent with an outbreak among sled dogs in Greenland. Bohm et al. (1989) hypothesized that arctic fox were responsible for carrying the virus from Canada to Greenland similar to the facilitation of rabies epizootics, which occur as infected fox cross the sea ice. This suggestion was based on sea ice conditions at the time, the observation that settlements in which CDV cases occurred were geographically isolated from one another, and that previous studies demonstrated fox migration from Canada to Greenland. An epizootic of CDV occurred in Kotzebue, Alaska during the winter of 1996-1997 killing approximately 200-300 dogs in the region. This strain was demonstrated to differ significantly from vaccine strains (Rockborn and Onderstepoort) with 19 nucleotide changes, 13 of which were shared with 7 other virulent field strains. This sequence determined to be most closely related to an isolate from a Siberian seal (99.4%) during the 1987-1988 epizootic in Lake Baikal. Although arctic fox do not live near Lake Baikal, there are other carnivores present in the region capable of long distance travel and potential dissemination of CDV (e.g. grizzly bears, wolves and red fox).

3.5.4 Concerns for Climate Change Impacts on Morbillivirus at the Marine-Terrestrial Interface

The arctic fox population in northern Alaska follows the cyclic abundance of lemmings and other rodents. Populations of arctic fox flourish when these prey are abundant and decline when prey populations decline, due to food availability. Arctic fox use sea ice more extensively for forage (e.g. seal carrion) in years when winter foods are limited, for example in low lemming years. Pamperin et al. (2008) followed collared individuals and suggested that diets for arctic fox were likely 100% marine while on the sea-ice given their consistent locations at distances that would preclude periodic trips to shore to feed on terrestrial resources. Rabies outbreaks in arctic fox populations occur in Alaska approximately every 3-5 years during the winter months (Ritter, 1981) when rodent availability is lowest and access to birds (and eggs) as alternative prey is lacking. Ritter (1981) hypothesized that traveling over long distances, congregation at food sources, and scavenging on infected carcasses in combination with nutritional stress may facilitate such outbreaks. The same forces may also drive epizootics of CDV, although whether the virus is endemic in arctic fox populations is unknown.

There is concern that diminishing sea ice may lead to increased presence of foxes and polar bears at human settlements (e.g. landfills and whale carcass remains) and industrial sites where anthropogenic food sources are present. This may facilitate greater interaction with domestic dogs and disease transmission. For density dependent disease, the presence of anthropogenic food sources such as baiting and waste stations that concentrate animals

in a given area can greatly enhance transmission rates (Wobeser, 2006). Polar bears and arctic fox congregated at anthropogenic food sources are likely to have more intraspecific and interspecific interactions. Significant differences were found for risk of CDV seropositivity for polar bears by age class with bears ages 5-7 most likely to be seropositive (Kirk et al., 2010), however we could not perform the same assessment for arctic fox because we were only successful in capturing adult animals.

Climate change is also expected to impact the long-range transport and fate of contaminants in the Arctic through alteration in ocean and air currents. Persistent organic pollutants (POPs) volatilize from lower latitudes and are transported to the Arctic where they are then deposited via precipitation and through other mechanisms (Lohmann et al., 2007). Locally, melting of glaciers may directly increase contaminant levels entering the food chain as contaminants deposited during their years of historical use are released (Blais et al., 2001). Permafrost has acted as a sink for POPs, and as it melts the contaminants are released (Suk et al., 2004). Reduced marine ice cover will increase ocean-atmosphere gas exchange and therefore deposition of contaminants like polychlorinated biphenyls (PCBs) and toxaphene, which are still entering the Arctic Ocean via the atmosphere (AMAP, 2003).

Indirect effects may occur as apex predators change prey species. For example, increasing contaminant loads have been documented in some polar bear sub-populations, possibly as a result of dietary shifts due to declining sea-ice (McKinney et al., 2009).

Stressors including decreasing access to optimal prey species, heat stress, exposure to increasing levels of contaminants and increasing burdens of infectious agents may act in synergism to decrease immunological resilience, thereby increasing susceptibility to morbillivirus infection. Changes in morbillivirus prevalence that may occur with climate change could pose an increased threat to the health of local people living along the coast and to wildlife if populations of prey bases and subsistence species are adversely impacted by morbillivirus epizootics.

3.5.5 Considerations for Origins of the Epizootic

Based on these data, the source of morbillivirus for arctic fox appears terrestrial at the genetic level, but serology suggests it to be of equivocally marine or terrestrial origin. The morbillivirus that infected arctic fox in 2007 may have entered the population from the marine environment (e.g. scavenging on infected seal carcasses), from interaction with terrestrial species (wildlife or domestic), or may be enzootic to this or neighboring populations of arctic fox, red fox or polar bears. It is of interest that a coinfection of rabies and distemper was diagnosed in one red fox (N=8) during the mortality event. Unfortunately, this animal was heavily scavenged and thus we did not have suitable tissue available for RT-PCR. Red foxes were not targeted in the capture studies and thus serological status was not assessed.

Using serology alone, we cannot determine whether polar bears are infected with or exposed to a single strain of morbillivirus, multiple morbilliviruses or perhaps carry a

distinct strain enzootic to southern Beaufort Sea polar bears in and/or the Arctic. In order for virus to have been detected in live captured arctic fox and polar bears, animals needed to have been sampled during active infection. It is possible that antibody presence in polar bears results from exposure only, with no significant resulting viremia or a viremia of no consequence. Alternatively, the virus may be able to replicate within polar bears without adverse effects, yet they may serve as a reservoir for other sympatric carnivores. This phenomenon has been observed among some seal species (Duignan et al., 1997). It is also possible that antibodies found in live captured fox resulted from an exposure prior to the documented epizootic, which afforded their survival (e.g. these animals were immunoprotected).

Polar bears and arctic fox may be exposed to different morbilliviruses where their ranges do not overlap. For example, southern Beaufort Sea polar bears may travel over 50 km a day (Amstrup, 2003), while foxes generally maintain smaller, defined home ranges extending farther onshore. In addition, foxes presently spend more time in close proximity to humans and anthropogenic food sources as compared to polar bears. They are commonly observed near dump-sites, outside kitchen facilities at oil camps, and accept handouts from humans. Transmission of morbillivirus between the two species at shared food resources (e.g. whale and seal carcasses) may be prevented by viral dessication due to the harsh Arctic climate. Furthermore, the virus infecting arctic fox resulted in mortality whereas the morbillivirus to which polar bears have been exposed does not appear to be lethal at a detectable scale and the biological effects of exposure

remain largely unknown. We do not know if polar bears actually become infected with the virus however hematology data collected for the southern Beaufort Sea polar bears (Kirk et al., 2010) demonstrate a significant negative association between specific white blood cell types and increasing titer to CDV.

3.6 CONCLUSION

Although morbillivirus in arctic fox appears canine (terrestrial) at the genetic (phosphoprotein) level, it cannot be distinguished from marine strains using serology. Viral RNA has not been successfully isolated from polar bears to date though serology suggests morbillivirus circulating among polar bears is distinct from that which infected sympatric arctic foxes in 2007. However, without viral RNA sequences from polar bears, seals, or other sympatric species of arctic fox, we are unable to determine which cross species transmissions of morbillivirus may be occurring in northern Alaska. The results of this study underscore the limitations of using serology alone to examine morbillivirus epidemiology. Phylogenetic analyses however do suggest that the strain is distinct from European, Asian, Eurasian, and North American field strains and most closely related to "arctic isolates" of CDV. Future studies should include not only obtaining morbilliviral nucleic acid from other carnivores across the Arctic, but also sequencing the less conserved H and fusion (F) genes to gain a better understanding of phylogeny of morbillivirus in the Arctic.

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3.8 REFERENCES

Aexander KA, Appel MJ. 1994. African wild dogs (*lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the masai mara national reserve, Kenya. Journal of Wildlife Diseases 30(4):481-485.

AMAP. 2003. AMAP Assessment 2002: The Influence of global change on contaminant pathways to, within, and from the Arctic, Oslo, Norway:Arctic Monitoring and Assessment Programme (AMAP).

Amstrup SC. 2003. Polar bear (*Ursus maritimus*). In: Wild Mammals of North America-Biology, Management, and Conservation, Feldhamer GA Thompson BC, Chapman JA (editors), Baltimore, MD: John Hopkins University Press, pp 587-610.

Amstrup SC, McDonald TL, Durner GM. 2004. Using satellilte radiotelemetry data to delineate and manage wildlife populations. Wildlife Society Bulletin 32(3):661–679.

Amstrup SC, Marcot BG, Douglas DC. 2008. A Bayesian network modeling approach to forecasting the 21st century worldwide status of polar bears. In: Arctic sea Ice Decline: Observations, Projections, Mechanisms, and Implications. Geophysical Monograph Series 180, DeWeaver ET, Bitz CM, Tremblay LB (editors), Washington, DC: American Geophysical Union, pp 213-268.

Alexander KA, Appel MJ. 1994. African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. Journal of Wildlife Diseases 30(4):481-485.

Appel MJG. 1987. Canine distemper virus. In: Virus infections of carnivores, Vol. 1, Appel MJG (editors), Amsterdam, The Netherlands: Elsevier Science Publishers, pp 133-159.

Ballard WB, Follmann EH, Ritter DG, Robards MD, Cronin MA. 2001. Rabies and canine distemper in an arctic fox population in Alaska. Journal of Wildlife Diseases 37(1):133-137.

Barrett T, Visser IKG, Mamaev L, Van Bressem MF, Osterhaus, ADME. 1993. Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology 193(2):1010-1012.

Barrett T, Blixenkrone-Møller M, Di Guardo G, Domingo M, Duignan P, Hall A, Mamaev L, Osterhaus AD. 1995. Morbilliviruses in aquatic mammals: report on round table discussion. Veterinary Microbiology 44(2-4):261-265.

Bentzen TW, Follmann EH, Amstrup SC, York GS, Wooler MJ, Muir DCG, O'Hara TM. 2008. Dietary biomagnification of organochlorine contaminants in Alaskan polar bears. Canadian Journal of Zoology 86(3):177-191.

Blais JM, Schindler DW, Muir DCG, Sharp M, Donald D, Lafrenière M, Braekevelt E, Strachan WMJ. 2001. Melting Glaciers: A major source of persistent organochlorines to subalpine Bow Lake in Banff National Park, Canada. Ambio 30(7):410-415.

Blixenkrone-Møller M, Svansson V, Have P, Bøtner A, Nielsen J. 1989. Infection studies in mink with seal-derived morbillivirus. Archives of Virology 106(1-2):165-170.

Bohm J, Blixenkrone-Møller M, Lund E. 1989. A serious outbreak of canine distemper among sled-dogs in northern Greenland. Arctic Medical Research 48(4):195-203.

Burek KA, Gulland FMD, O'Hara TM. 2008. Effects of climate change on Arctic marine mammal health. Ecological Applications 18(2) Supplement:S126-134.

Campbell D, Welch K, Baker C, Overell J. 2007. Canine Distemper Virus in Sled Dogs-Nunavut and NT. Canadian Cooperative Wildlife Health Centre Newsletter 13(1):8-9.

Cattet MRL, Duignan PJ, House CA, St. Aubin, DJ. 2004. Antibodies to canine distemper and phocine distemper viruses in polar bears from the Canadian Arctic. Journal of Wildlife Diseases 40(2):338–342.

Chomel BB, Kasten RW Chappuis G, Soulier M, Kikuchi Y. 1998. Serological survey of selected canine viral pathogens and zoonoses in grizzly bears (*Ursus arctos horribilis*) and black bears (*Ursus americanus*) from Alaska. Revue Scientifique et Technique 17(3):756-766.

Cleaveland S, Appel MGJ, Chalmers WSK, Chillingworth C, Kaare M, Dye C. 2000. Serological and demographic evidence for domestic dogs as a source of infection for Serengeti wildlife. Veterinary Microbiology 72(3-4):217-227.

Comiso JC. 2006. Abrupt decline in the arctic winter sea ice cover. Geophysical Research Letters 33: L18504, doi: 10.1029/2006GL027341 [Online August 21, 2006].

Deem SL, Spelman LH, Yates RA, Montali RJ. 2000. Canine distemper in terrestrial carnivores: a review. Journal Zoo and Wildlife Medicine 31(4):441–451.

Duignan PJ, Nielsen O, House C, Kovacs KM, Duffy N, Early G, Sadove S, St. Aubin DJ, Rima K, Geraci JR .1997. Epizootiology of morbillivirus in harp, hooded, and ringed seals from the Canadian Arctic and western Atlantic. Journal of Wildlife Diseases 33(1):7-19.

Follmann EH, Garner GW, Evermann JF. 1996. Serological evidence of morbillivirus infection in polar bears (*Ursus maritimus*) from Alaska and Russia. The Veterinary Record 138(25):615-618.

Garner GW, Evermann JF, Saliki JT, Follmann EH, McKeirnan AJ. 2000. Morbillivirus Ecology in Polar Bears (*Ursus maritimus*). Polar Biology 23(7):474-478.

Goldstein T, Mazet JAK, Verena GA, Doroff AM, Burek KA, Hammond JA. 2009. Emerging Infectious Diseases 15(6):925-927.

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5):696-704.

Hammond JA, Pomeroy PP, Hall AJ, Smith VJ. 2002. Identification and real-time PCR quantification of phocine distemper virus from two colonies of Scottish grey seals in 2002. Journal of General Virology 86(9):2563-2567.

Harder TC, Osterhaus ADME. 1997. Canine distemper virus-a morbillivirus in search of new hosts? Trends in Microbiology 5(3):120-124.

Harkonen T, Dietz R, Reijnders P, Teilmann J, Harding K, Hall A, Brasseur S, Siebert U, Goodman SJ, Jepson PD, Dau Rasmussen T, Thompson P. 2002. A review of the 1988 and 2002 phocine distemper virus epidemics in European harbour seals. Diseases of Aquatic Organisms 68(2):115-130.

Holland MM, Bitz CM, Tremblay B. 2006. Future abrupt reductions in the summer Arctic sea ice, Geophysical Research Letters 33: L23503; doi:10.1029/2006GL028024 [Online December 6, 2006].

Hughes-Hanks JM, Rickard LG, Panuska C, Saucier JR, O'Hara TM, Dehn L, Rolland RM. 2005. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in five marine mammal species. Journal of Parasitology 91(5)1225-1228.

Kirk CM, Amstrup S, Swor R, Holcomb D, O'Hara TM. 2010. Hematology of southern Beaufort Sea polar bears (2005-2007): Biomarker for an Arctic ecosystem health sentinel. EcoHealth doi:10.1007/S10393-010-0322 [Online July 7, 2010].

Kraft AE, Lichy JH, Lipscomb TP, Klaunberg BA, Kennedy S, Taubenberger JK. 1995. Postmortem diagnosis of morbillivirus infection in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic and Gulf of Mexico epizootics by a polymerase chain reaction based assay. Journal of Wildlife Disease 31(3):410-415.

Kuiken T, Kennedy S, Barrett T, Borgsteede F, Deaville R, Duck C, Eybatov T, Forsyth M, Foster G, Jepson P, Kydyrmanov A, Mitrofanov I, van de Bildt M, Ward C, Wilson S, Osterhaus ADME. 2006. The 2000 canine distemper epidemic in Caspian seals (*Phoca caspica*): pathology and analysis of contributory factors. Veterinary Pathology 43(3):321-338.

Lohmann R, Breivik K, Dachs J, Muir D. 2007. Global Fate of POPs: Current and future research directions. Environmental Pollution 150(1):150–165.

Maes RK, Wise AG, Fitzgerald SD, Ramudo A, Kline J, Vilnis A, Benson B. 2003. A canine distemper outbreak in Alaska: diagnosis and strain characterization using sequence analysis. Journal of Veterinary Diagnostic Investigation 15(3):213-220.

Mamaev LV, Denikina NN, Belikov SI, Volchkov VE, Visser IK, Fleming M, Kai C, Harder TC, Liess B, Osterhaus AD, Barrett T. 1995. Characterization of morbilliviruses isolated from Lake Baikal seals (*Phoca sibirica*). Veterinary Microbiology 44(4):251–259.

McCarthy AJ, Shaw MA, Goodman SJ. 2007. Pathogen evolution and disease emergence in carnivores. Proceedings of the Royal Society B: Biological Sciences 274(1629):3165-3174.

McKinney MA, Peacock E, Letcher RJ. 2009. Sea ice associated diet change increases the levels of chlorinated and brominated contaminants in polar bears. Environmental Science &Technology 43(12):4334-4339.

Pamperin NJ, Follmann EH, Person BT. 2008. Sea-ice use by arctic foxes in northern Alaska. Polar Biology 31(11):1432-2056.

Pardo ID, Johnson GC, Kleiboeker SB. 2005. Phylogenetic characterization of canine distemper viruses detected in naturally infected dogs in North America. Journal of Clinical Microbiology 43(10):5009-5017.

Philippa JD, Leighton FA, Daoust PY, Nielsen O, Pagliarulo M, Schwantje H, Shury T, Van Herwijnen R, Martina BEE, Kuiken T, Van de Bildt MWG, Osterhaus ADME. 2004. Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. The Veterinary Record 155(5):135-140.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9):817-818.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Systematic Biology 53(5):793-808.

Rausch RL. 2003. Cystic Echinococcus in the Arctic and Sub-Arctic. Parasitology 127 Supplement:S73-85.

Rima BK, Wishaupt RG, Welsh MJ, Earle JA. 1995. The evolution of morbilliviruses: A comparison of nucleocapsid gene sequences including a porpoise morbillivirus. Veterinary Microbiology 44(2-4):127–134.

Ritter D. 1981. Rabies. In: Alaskan Wildlife Diseases, Dieterich RA (Editor), Fairbanks, Alaska: University of Alaska, pp 6-12.

Ross PS. 2002. The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. Human and Ecological Risk Assessment 8(2):277-292.

Saliki JT, Cooper EJ, Gustavson JP. 2002. Emerging morbilliviral infections of marine mammals - development of two diagnostic approaches. Annals of The New York Academy of Sciences 969(1):51-59.

Stirling I, Spencer C, Andriashek D. 1989. Immobilization of polar bears (*Ursus maritimus*) with Telazol® in the Canadian Arctic. Journal of Wildlife Diseases 25(2):159-168.

Stirling I, Øritsland NA. 1995. Relationships between estimates of ringed seal and polar bear populations in the Canadian Arctic. Canadian Journal of Fisheries and Aquatic Sciences 52(12):2594–2612.

Stirling I, Lunn NJ. 1997. Environmental fluctuations in arctic marine ecosystems as reflected by variability in reproduction of polar bears and ringed seals. In:Woodin SJ, Marquiss M (editors), Ecology of Arctic Environments. Special Publication (13) of the British Ecological Society, Blackwell Science Ltd., Oxford, England, pp 167-181.

Stirling I, Lunn NJ, Iacozza J. 1999. Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. Arctic 52(3):294-306.

Suk WA, Avakian MD, Carpenter D, Groopman JD, Scammell M, Wild CP. 2004. Human exposure monitoring and evaluation in the Arctic: The importance of understanding exposures to the development of public health policy. Environmental Health Perspectives 112(2):113–120.

TRI Reagent - RNA, DNA, protein isolation reagent. Manufacturer's protocol. 1995. Molecular Research Center, Inc. Cincinnati, OH.

Tryland M, Neuvonen E, Huovilainen A, Tapiovaara H, Osterhaus ADME, Wiig Ø, Derocher AE. 2005. Serologic survey for selected virus infections in polar bears at Svalbard. Journal of Wildlife Diseases 41(2):310–316.

U.S. Fish and Wildlife Service. 2008. Endangered and threatened wildlife and plants; determination of threatened status for the polar bear (*Ursus maritimus*) throughout its range. Federal Register 73(95):28212–28303.

van de Bildt MWG, Kuiken T, Osterhaus ADME. 2005. Cetacean morbilliviruses are phylogenetically divergent. Archives of Virology 150(3): 577–583.

Wobeser GA. 2006. Essentials of Disease in Wild Animals, Ames, IA: Wiley-Blackwell Publishing.

Zarnke RL, Ver Hoef JM; DeLong RA. 2004. Serologic survey for selected disease agents in wolves (*Canis lupus*) from Alaska and the Yukon Territory, 1984–2000. Journal of Wildlife Diseases 40(4):632-636.

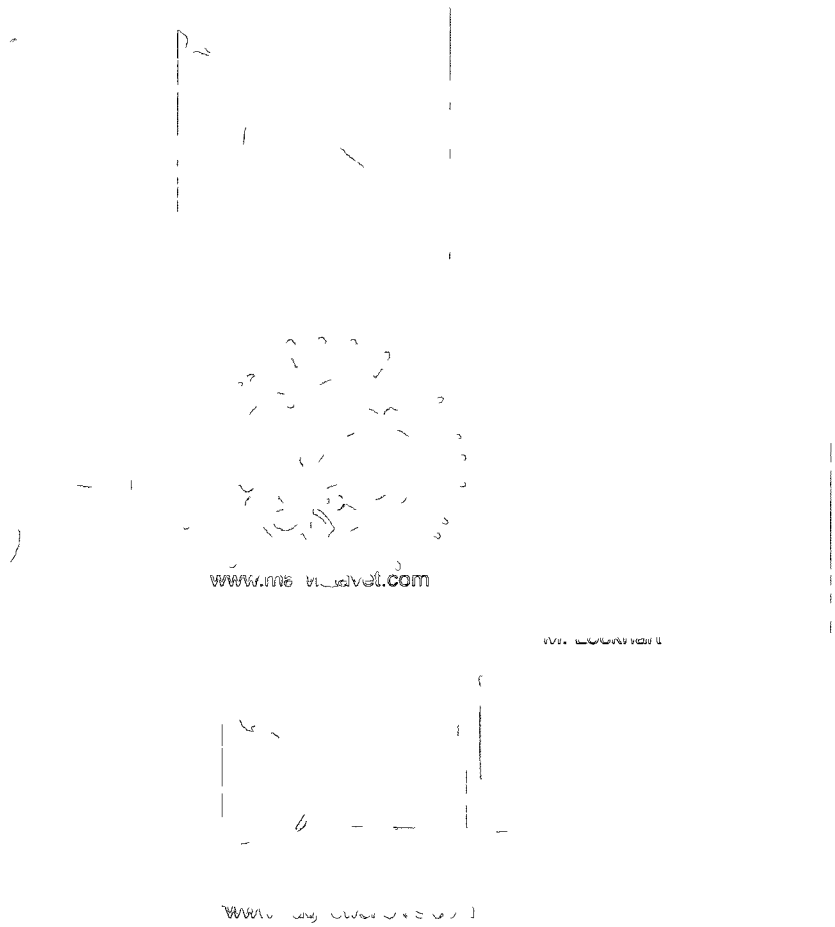

Figure 3.1 Conceptual model for hypothesized morbillivirus transmission in northern Alaska.

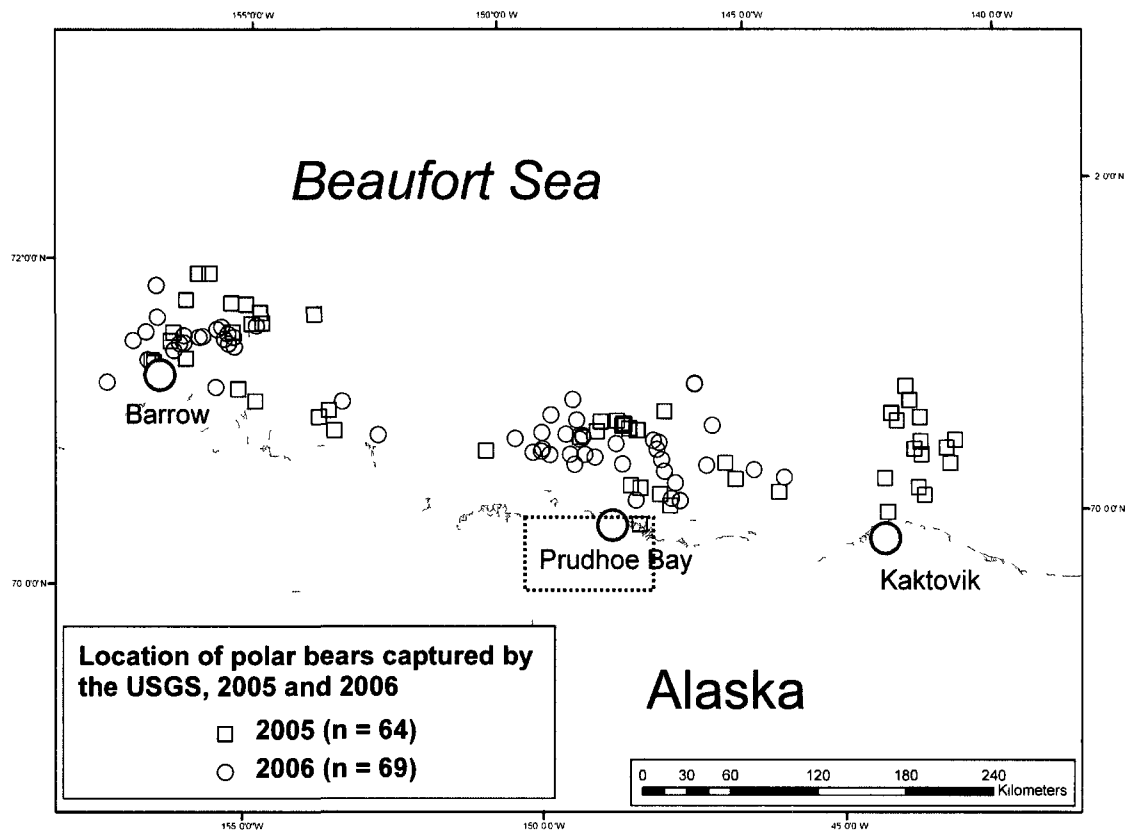

Figure 3.2 Map of northern Alaska showing area of southern Beaufort Sea polar bear (2005-2006) and Prudhoe Bay area arctic fox (2007) captures. Individual capture locations for arctic fox are not included on the map but trapping area is denoted by the rectangle.

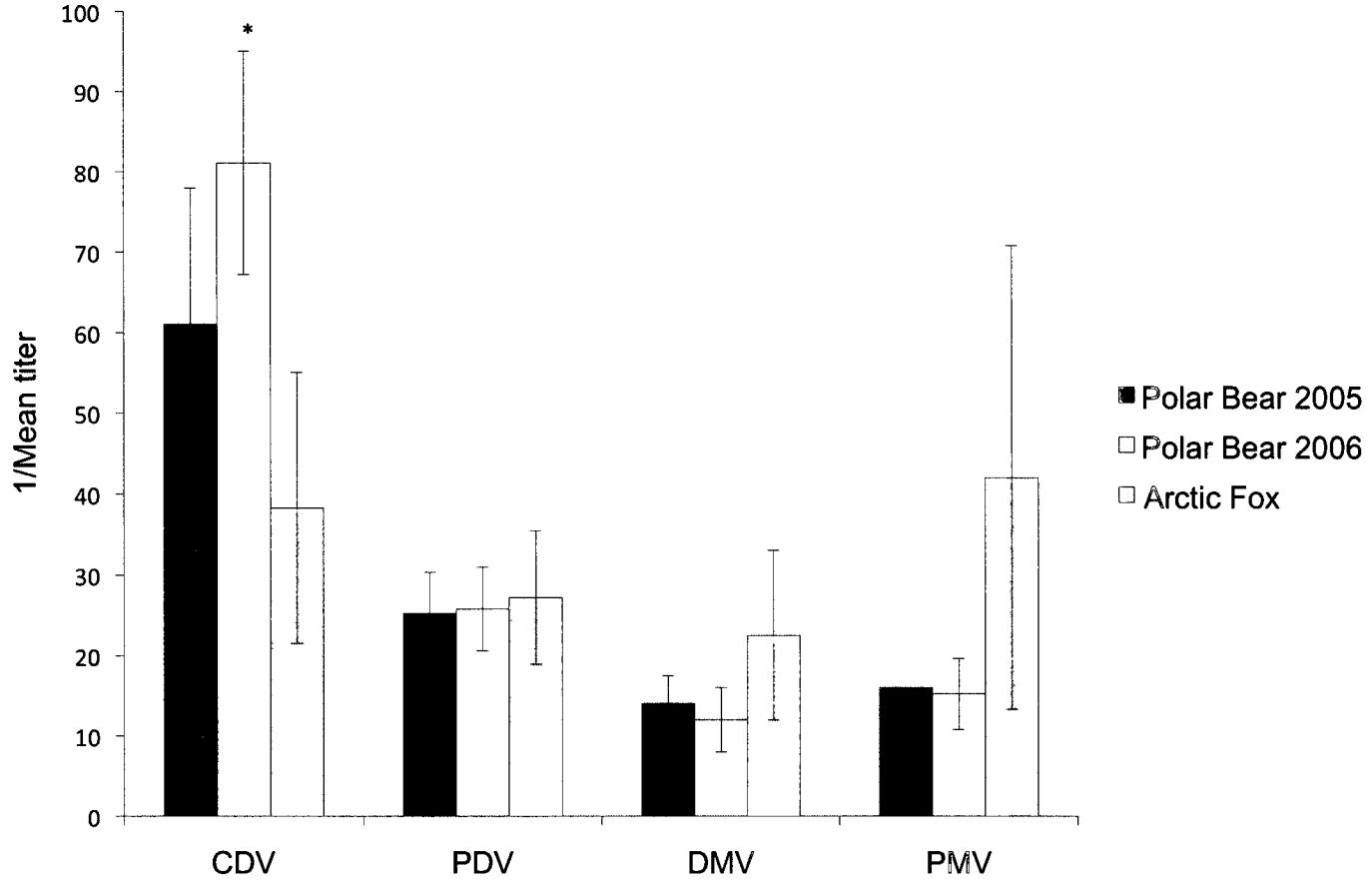

Figure 3.3 1/Mean titer (± SE) for southern Beaufort Sea polar bears (2005-2006) and Prudhoe Bay area arctic fox (2007), northern Alaska. *Denotes significant difference in CDV prevalence ($p \leq 0.05$) between groups based on the Wilcoxon signed-rank test.

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Ondesterpoort_Vaccine             GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Rockborn_Vaccine                  GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTCCCTGCAGG 50
Dog_Kazhakstan__2007              GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Caspian_Seal_Kazakhstan__2007     GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Siberian_Seal_Russia__1988        GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
_Dog_Alaska__USA_1996-97          ------------------------------------------CCTGCAGG 8
Arctic.Fox_Alaska_USA__2007       GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Dog_18133_USA__2004               ------------------------------------------CCTGCAGG 8
Ferret_Germany_1989               GAAGAGATTAAGGGAATCGAAGATGCTGACAGCCTCGTGGTACCTGCAGG 50
Dog_Germany_1993                  GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Dog_5804_Germany__1990            GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Lynx_Canada__2008                 -AAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 49
Dog_A75-15_USA_1975               GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Dog_Jujo_Japan                    GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Dog_Yanaka_1992-94                GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
Dog_Hamamatsu_Japan_1992-94       GAAGAGGTTAAGGGAATCGAAGATGCTGACAGTCTCGTGGTACCTGCAGG 50
PDV-1                             GAAAAGGTTAAGGGAATCGAAGATGCTGACAGTCTCATGGTACCAGCAGG 50
CMV                               ------GTTGAAGGAGTCAAGGATGCTGACCTGCTCGTGGTTCCAACAGG 44
DMV                               CAAGCGGTTGAAGGAGTCAAGGATGCTGACCTGCTCGTGGTTCCAACAGG 50
                                                                                 **  **** 

Ondesterpoort_Vaccine             CACTGTCGGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Rockborn_Vaccine                  CACTGTCGGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Dog_Kazhakstan__2007              CACTGTCGGTAATCGAGGATTCGAGAGCGGAGAAGGAAGCCCTGATGATA 100
Caspian_Seal_Kazakhstan__2007     CACTGTCGGTAATCGAGGATTCGAGAGCGGAGAAGGAAGCCCTGATGATA 100
Siberian_Seal_Russia__1988        CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
_Dog_Alaska__USA_1996-97          CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 58
Arctic.Fox_Alaska_USA__2007       CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Dog_18133_USA__2004               CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 58
Ferret_Germany_1989               CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAGGGAAGCCTTGATGATA 100
Dog_Germany_1993                  CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Dog_5804_Germany__1990            CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Lynx_Canada__2008                 CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 99
Dog_A75-15_USA_1975               CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Dog_Jujo_Japan                    CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Dog_Yanaka_1992-94                CGCTGTCAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
Dog_Hamamatsu_Japan_1992-94       CGCTGTTAGTAATCGAGGATTCGAGAGAGGAGAAGGAAGCCTTGATGATA 100
PDV-1                             CCCTCCAAGTAATAGGGGATTCGAAGGAAGAGAGGGAAGCCTTGATGATA 100
CMV                               CAGTGATGATGATGCAGAATTCAGAGACGGAGATGAGAGCTCTCTCGAGA 94
DMV                               CAGTGATGATGATGCAGAATTCAGAGACGGAGATGAGAGCTCTCTCGAGA 100
                                  *  *     * **   * ****       **** *  ***  *   ** *  

Ondesterpoort_Vaccine             GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Rockborn_Vaccine                  GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Dog_Kazhakstan__2007              GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Caspian_Seal_Kazakhstan__2007     GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Siberian_Seal_Russia__1988        GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
_Dog_Alaska__USA_1996-97          GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 108
Arctic.Fox_Alaska_USA__2007       GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Dog_18133_USA__2004               GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 108
Ferret_Germany_1989               GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Dog_Germany_1993                  GCACTGAGGATTCTGGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
Dog_5804_Germany__1990            GCACTGAGGATTCTGGCGAAGATTATTCCGAGGGAAATGCTTCATCTAAC 150
Lynx_Canada__2008                 GCACTGAGGATTCTGGCGAAGATTATTCCGAGGGAAATGCTTCATCTAAC 149
Dog_A75-15_USA_1975               GCACTGAGGATTCTGGCGAAGATTATTCCGAGGGAAATGCTTCATCTAAC 150
Dog_Jujo_Japan                    GCACTGAAGATTCTGGCGAAGATTATTCCGAGGGAAATGCTTCATCTAAC 150
Dog_Yanaka_1992-94                GCACTGAGGATTCTGGCGAAGATTATTCCGAGGGAAATGCTTCATCTAAC 150
Dog_Hamamatsu_Japan_1992-94       GCACTGAGGATTCTGGCGAAGATTATTCCGAGGGAAATGCTTCATCTAAC 150
PDV-1                             GCATTGAAGATTCTAGCGAAGATTATTCCGAAGGAAATGCTTCATCTAAC 150
CMV                               GCGATGGTGAATCTGGCACTGTTGATACCAGAGGAAATTCTTCCTCTAAC 144
DMV                               GCGATGGTGAATCTGGCACTGTTGATACCAGAGGAAATTCTTCCTCTAAC 150
                                  **  **  ** *** **   * * ** **   ****** **** ******
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Figure 3.4 Nucleotide sequence alignment of a 389 bp P gene fragment of sequences included in phylogenetic analyses. Nucleotides identical to the consensus are shown in dot and missing nucleotides are designated by dashes. Sequences for the Alaska sled dog and Missouri dog case 18133 are 42 bp shorter than the Arctic Fox sequence and CMV is 6 bp shorter.

```
Ondesterpoort_Vaccine             TGGGGATATTCTTTCGGCCTTAAACCGGACAGAGCAGCTGATGTGAGCAT 200
Rockborn_Vaccine                  TGGGGATATTCTTTCGGCCTTAAACCGGACAGAGCAGCTGATGTGAGCAT 200
Dog_Kazhakstan__2007              TGGGGATATTCTTTCGGCCTTAAACCAGACAGGGCAGCTGATGTGAGCAT 200
Caspian_Seal_Kazakhstan__2007     TGGGGATATTCTTTCGGCCTTAAACCAGACAGGGCAGCTGATGTGAGCAT 200
Siberian_Seal_Russia__1988        TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 200
_Dog_Alaska__USA_1996-97          TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 158
Arctic.Fox_Alaska_USA__2007       TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 200
Dog_18133_USA__2004               TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 158
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Dog_Germany_1993                  TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 200
Dog_5804_Germany__1990            TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 200
Lynx_Canada__2008                 AGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 199
Dog_A75-15_USA_1975               TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCAGCTGATGTGAGCAT 200
Dog_Jujo_Japan                    TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCGGCTGATGTGAGCAT 200
Dog_Yanaka_1992-94                TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCGGCTGATGTGAGCAT 200
Dog_Hamamatsu_Japan_1992-94       TGGGGATATTCTTTCGGCCTTAAACCAGACAGAGCGGCTGATGTGAGCAT 200
PDV-1                             TGGGGATATACTTTTGGCCTTAACCCAGACAGAGCAGCTGATGTGAGCAT 200
CMV                               AGGGGATCTGCTCCCAGGATTAAGGTCGAGAGATCTGCTGACGTTGAGAC 194
DMV                               AGGGGATCTGCTCCCAGGATTAAGGTCGAGAGATCGTCTGACGTTGAGAC 200
                                   ****** * **    *  ****    ** **  *  **** **    *

Ondesterpoort_Vaccine             GCTGATGGAAGAGGAATTAAGTGCTCTACTCAG----GACAAGCAGAAAT 246
Rockborn_Vaccine                  GCTGATGGAAGAGGAATTAAGTGCTCTACTCAG----GACAAGCAGAAAT 246
Dog_Kazhakstan__2007              GCTGATGGAAGAGGAATTAAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
Caspian_Seal_Kazakhstan__2007     GCTGATGGAAGAGGAATTAAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
Siberian_Seal_Russia__1988        GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAG----GACAAGCAGAAAT 246
_Dog_Alaska__USA_1996-97          GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAG----GACAAGCAGAAAT 204
Arctic.Fox_Alaska_USA__2007       GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAG----GACAAGCAGAAAT 246
Dog_18133_USA__2004               GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAG----GACAAGCAGAAAT 204
Ferret_Germany_1989               GCTGATGGAAGAGGAATTGAGCGCTCTGCTCAG----GACAAGCAGAAGT 246
Dog_Germany_1993                  GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
Dog_5804_Germany__1990            GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
Lynx_Canada__2008                 GCTAATGGAAGAGGAATTGAGTGCTTTGCTCAA----GACAAGCAGAAAT 245
Dog_A75-15_USA_1975               GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAG----GACAAGCAGAAAT 246
Dog_Jujo_Japan                    GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
Dog_Yanaka_1992-94                GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
Dog_Hamamatsu_Japan_1992-94       GCTGATGGAAGAGGAATTGAGTGCTCTGCTCAA----GACAAGCAGAAAT 246
PDV-1                             GCTAATGGAGGAAGAGTTGACTACTCTGCTTGG----CACAGGCCACAAT 246
CMV                               TATAAGCAGTGAAGAGCTACAAGGACTGATTAGATCTCAGAGTCAAAAAC 244
DMV                               TATAAGCAGTGAAGAGCTACAAGGACTGATTAGATCTCAGAGTCAAAAAC 250
                                    * *     ** **  *        *  *        * *  *   *

Ondesterpoort_Vaccine             GTAG-GGATTCAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACATAATC 295
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Dog_Kazhakstan__2007              GTAG-GGATTCAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACATAATC 295
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Siberian_Seal_Russia__1988        GTAG-GGATTCAGAAAAGGGAGGGGAAAACTCTGCAGTTCCCACACAATC 295
_Dog_Alaska__USA_1996-97          GTAG-GGATTCAGAAAAGGGAGGGGAAAACTCTGCAGTTCCCACACAATC 253
Arctic.Fox_Alaska_USA__2007       GTAG-GGATTCAGAAAAGGGATGGGAAAACTCTGCAGTTCCCACACAATC 295
Dog_18133_USA__2004               GTAG-GGATTCAGAAAAGGGATGGGAAAACTCTGCAGTTCCCACACAATC 253
Ferret_Germany_1989               GTAG-GGATTCAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACACAATC 295
Dog_Germany_1993                  GTAG-GGATTCAGAAAAGGGATGGAAAGACTCTGCAGTTCCCACACAATC 295
Dog_5804_Germany__1990            GTAG-GGATTCAGAAAAGGGATGGAAAGACTCTGCAGTTCCCACACAATC 295
Lynx_Canada__2008                 GTAG-GGATACAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACACAATC 294
Dog_A75-15_USA_1975               GTAG-GGATACAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACGCAATC 295
Dog_Jujo_Japan                    GTGG-GGATTAAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACACAATC 295
Dog_Yanaka_1992-94                GTGG-GGATTAAGAAAAGGGATGGGAAGACTCTGCAGTTCCCACACAATC 295
Dog_Hamamatsu_Japan_1992-94       GTGG-GGATTAAGAAAAGGGATGGGATGACTCTGCAGTTCCCACACAATC 295
PDV-1                             GCCG-GGGGCCAAAAGAGGGACGGGAGAACTCTACAGTTTCCGAATAGTC 295
CMV                               ATAATGGATTTGGAGTAG--AC---AGATTCCTAAAGGTCCCAC-CAATT 288
DMV                               ATAATGGATTTGGAGTAG--AC---AGATTCCTAAAGGTCCCAC-CAATT 294
                                       **      *  **  *    *     **  ** * **    * *
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Figure 3.4 (Continued).

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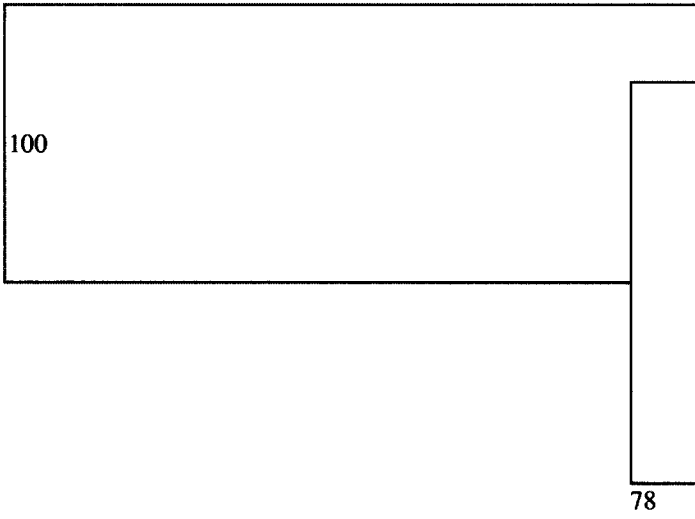
Ondesterpoort_Vaccine              CCGAAGGTAAGACAAG-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Rockborn_Vaccine                   CCGAAGGTAAGACAAG-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Dog_Kazhakstan__2007               CCGAAGGTAAGACAAG-GGTTCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Caspian_Seal_Kazakhstan__2007      CCGAAGGTAAGACAAG-GGTTCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Siberian_Seal_Russia__1988         CCGAAGGTAAGACAGG-AGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
_Dog_Alaska__USA_1996-97           CCGAAGGTAAGACAGG-AGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 302
Arctic.Fox_Alaska_USA__2007        CCGAAGGTAAGACAGG-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Dog_18133_USA__2004                CCGAAGGTAAGACAGG-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 302
Ferret_Germany_1989                CCGAAGGTAAGACAGG-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Dog_Germany_1993                   CCGAAGGTAAGACAGG-GGATCCGGAGTGTGGGTCCATTAAAAAGGGCAC 344
Dog_5804_Germany__1990             CCGAAGGTAAGACAGG-GGATCCGGAGTGTGGGTCCATTAAAAAGGGCAC 344
Lynx_Canada__2008                  CAGAAGGTAAGACAGG-GGATCCGGAGTGTGGTTCCATTAAAAAGGGCAC 343
Dog_A75-15_USA_1975                CAGAAGGTAAGACAGG-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Dog_Jujo_Japan                     CCGAAGGTAAGACAGA-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
Dog_Yanaka_1992-94                 CCGAAGGTAAGACAGA-GGATCCGGAGTGTGGACCCATTAAAAAGGGCAC 344

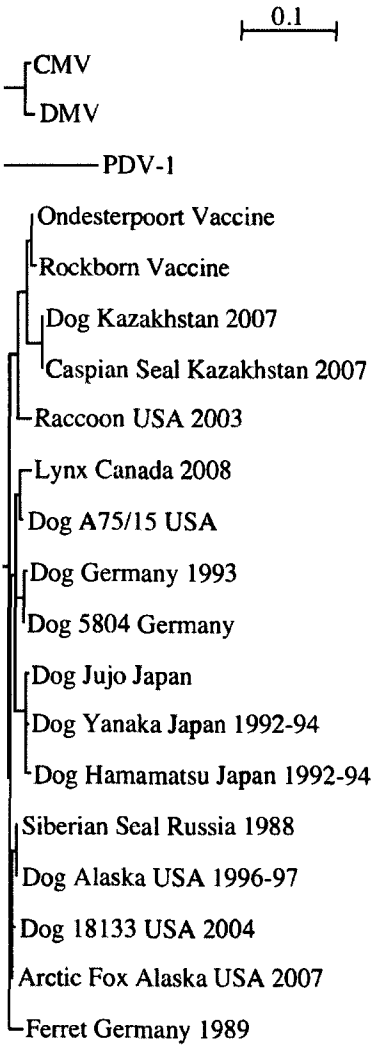
Dog_Hamamatsu_Japan_1992-94        CCGAAGGTAAGACAGA-GGATCCGGAGTGTGGATCCATTAAAAAGGGCAC 344
PDV-1                              CCGAAGGGAGTATAGG-GAACCAAGTATGCGAACCCATTAAAAAGGGCAC 344
CMV                                CCAACCTCCGTGCCGCTGGACCCCGCTTCCAAATCCATTAAAAAGGGCAC 338
DMV                                CCAACCTCAGTGCCGCTGGACCCCGCTCCCAAATCCATTAAAAAGGGCAC 344
                                   *  *                 *  *         ****************


Ondesterpoort_Vaccine              AGAAGAGAGGTCAGTCTCACATGGAATGGGGATAGTTGCTGGATC 389
Rockborn_Vaccine                   AGAAGAGAGGTCAGTCTCACATGGAATGGGGATAGTTGCTGGATC 389
Dog_Kazhakstan__2007               AGAAGAGAGGTCAGTCTCACAGGGAATGGGGATAGTTGCTGGATC 389
Caspian_Seal_Kazakhstan__2007      AGAAGAGAGGTCAGTCTCACAGGGAATGGGGATAGTTGCTGGATC 389
Siberian_Seal_Russia__1988         AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
_Dog_Alaska__USA_1996-97           AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 347
Arctic.Fox_Alaska_USA__2007        AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
Dog_18133_USA__2004                AGGAGAGAAGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 347
Ferret_Germany_1989                AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
Dog_Germany_1993                   AGGAGAGAGGTCAGCCTCACATGGAATGGAGATAGTTGCTGGATC 389
Dog_5804_Germany__1990             AGGAGAGAGGTCAGCCTCACATGGAATGGAGATAGTTGCTGGATC 389
Lynx_Canada__2008                  AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 388
Dog_A75-15_USA_1975                AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
Dog_Jujo_Japan                     AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
Dog_Yanaka_1992-94                 AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
Dog_Hamamatsu_Japan_1992-94        AGGAGAGAGGTCAGCCTCACATGGAATGGGGATAGTTGCTGGATC 389
PDV-1                              AGGAGAGAAGTTAGCCTCACATGGAATGATGACCGCTGCTGGATT 389
CMV                                AGGAGAGAGATCAGCCTTATCTGGGACGGTGACCGAGTTTTCATT 383
DMV                                AGGAGAGAGATCAGCCTTATCTGGGACGGAGACCGAGTTTTCATT 389
                                   ** *****  * ** ** *   ** * *  **  *    *  **
```

Figure 3.4 (Continued)

(a) Full Maximum Likelihood Tree

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CMV
DMV
PDV-1
Ondesterpoort Vaccine
Rockborn Vaccine
Dog Kazakhstan 2007
Caspian Seal Kazakhstan 2007
Raccoon USA 2003
Lynx Canada 2008
Dog A75/15 USA
Dog Germany 1993
Dog 5804 Germany
Dog Jujo Japan
Dog Yanaka Japan 1992-94
Dog Hamamatsu Japan 1992-94
Siberian Seal Russia 1988
Dog Alaska USA 1996-97
Dog 18133 USA 2004
Arctic Fox Alaska USA 2007
Ferret Germany 1989

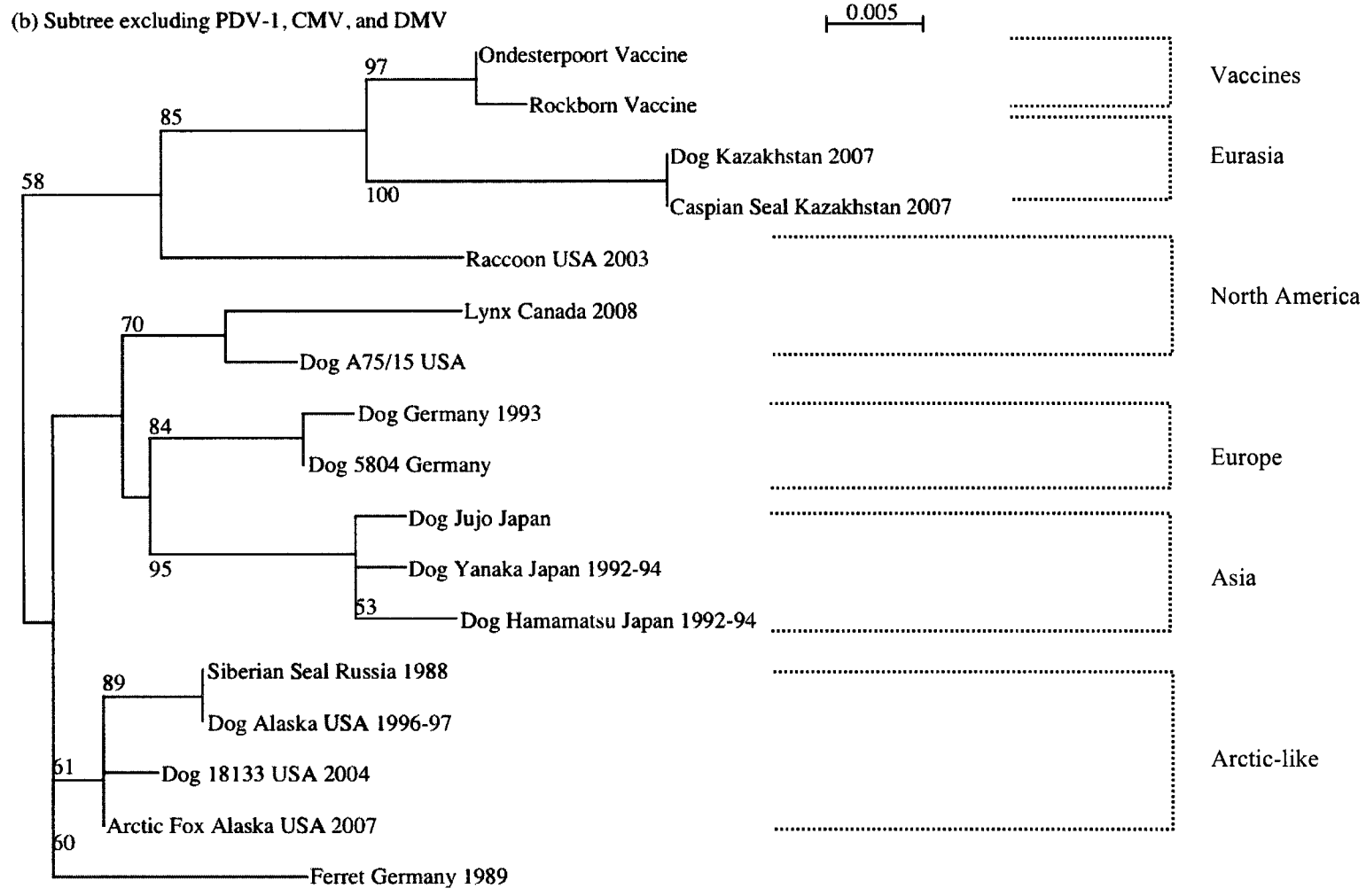

Figure 3.5 (Continued)

Table 3.1 (a) Morbillivirus seroprevalence (mean %, N, and 95% CI) for southern Beaufort Sea polar bears (2005-2006) and Prudhoe Bay area arctic fox (2007), northern Alaska as determined by Oklahoma Animal Disease Diagnostic Laboratory (OADDL) and University of Georgia, Athens (UGA). (b) Matrix displaying the number of arctic fox seropositive by morbillivirus for both laboratories where OADDL: DMV + PMV = UGA: CMV.

(a)

	Polar Bear				Arctic Fox			
	OADDL				OADDL		UGA	
	Sample Year (N)				Sample Year (N)			
	2005 (63)	95% CI	2006 (73)	95% CI	2007 (11)	95% CI	2007 (11)	95% CI
CDV	49.2% (31)	36.4%-62.1%	50.7% (37)	38.7%-62.6%	63.6% (7)	30.8%-89.1%	90.9% (10)	58.7%-99.8%
PDV	20.6% (13)	11.5%-32.7%	27.4% (20)	17.6%-39.1%	90.9% (10)	58.7%-99.8%	81.8% (9)	48.2%-97.7%
DMV	6.3% (4)	1.8%-15.5%	2.7% (2)	0.3%-9.5%	72.7% (8)	39%-94%	NT	NT
PMV	1.6% (1)	0%-8.5%	6.8% (5)	2.3%-15.3%	36.4% (4)	10.9%-69.2%	NT	NT
CMV	NT	NT	NT	NT	NT		81.8% (9)	48.2%-97.7%

(b)

OADDL					UGA			
# Arctic Fox	**CDV**	**PDV**	**DMV**	**PMV**	**# Arctic Fox**	**CDV**	**PDV**	**CMV**
3	+	+	+	+	8	+	+	+
2	+	+	+	−				
1	−	+	+	+				
2	+	+	−	−	1	+	+	−
					1	+	−	+
2	−	+	+	−				
1	−	−	−	−	1	−	−	−

CHAPTER 4

Molecular Ecology of *Echinococcus* and Taeniinae in Arctic Fox (*Vulpes lagopus*) of Northern Alaska: Characterization of the nad1 gene fragment[1]

4.1 ABSTRACT

The family Cyclophyllidean tapeworms are the most prevalent cestode parasites of terrestrial vertebrates. Human infections of *Echinococcus,* a genus within this group, can result in significant morbidity and mortality rates of up to 80% when untreated. Although well studied in other regions of Alaska, most on notably St. Lawrence Island, the distribution and abundance of *Echinococcus* species on the North Slope (Arctic regions north of the Brooks Range Mountains) of Alaska are poorly known. Climate change, industrial encroachment, increasing human population size, and changing dynamics of canid host populations (arctic fox, red fox, domestic dogs, wolves) are likely to impact risk factors for parasitic infection in the Arctic. Therefore, understanding current ecology and monitoring prevalence will be necessary to understand the human health consequences of ongoing and projected changes in the Arctic. We propose that canid definitive hosts provide the best sentinel for monitoring alterations in *Echinococcus* ecology and potential icreases in infection pressure to humans. In addition, we characterized a fragment of the mitochondrial nad1 gene of

[1] Prepared for submission in the style of EcoHealth: Kirk CM, Ballweber L, Follman E, O'Hara TM. 2010. Molecular Ecology of *Echinococcus* and Taeniinae in Arctic Fox (*Vulpes lagopus*) of Northern Alaska: Characterization of the nad1 gene fragment.

Echinococcus, which also amplified two species of *Taenia,* from North Slope definitive hosts (arctic fox and sled dogs). We unequivocally identified the species of *Echinococcus* in this region as *Echinococcus multilocularis* and simultaneously detected isolates of *Taenia crassiceps* and *Taenia polyacantha*. Maximum likelihood analysis revealed that the North Slope isolate of *Echinococcus multilocularis* was most closely related to strains from China, consistent with the detection of Asian strains of the parasite in other regions of Alaska. Phylogenetic analyses for isolates of *Taenia crassiceps* and *Taenia polyacantha* demonstrated that isolates from the North Slope of Alaska were more similar to isolates from other regions of the Arctic than to isolates from subarctic regions of the state.

4.2 INTRODUCTION

Within the diverse assemblage of cyclophyllidean tapeworms is the family Taeniidae, which includes the closely related subfamilies Taeninae and Echinococcinae. Both occur as adults in the intestine of carnivorous and omnivorous mammals. Some of these organisms are of substantial medical (zoonotic) and veterinary significance, with respect to morbidity and mortality in humans and production losses to domestic food animals worldwide (Hoberg, 2002).

In Alaska, two species of *Echinococcus* occur: *E. granulosus* and *E. multilocularis.* Intermediate hosts for *E. granulosus* include moose (*Alces alces*), reindeer/caribou (*Rangifer tarandus*); and for *E. multilocularis*, include voles (*Microtus*) and lemmings (*Lemmus* and *Dicrostonyx).* For both species of *Echinococcus* canids (domestic or wild) serve as definitive hosts, however *E. granulosus* utilizes wolves (*Canis lupus*) (and possibly coyotes [*Canis latrans*]) whereas *E. multilocularis* utilizes arctic (*Vulpes lagopus*) and red foxes (*Vulpes vulpes*, Castrodale, 2003). These parasites are ecologically segregated due to predator-prey relationships of their hosts; however when the two species are sympatric, the final or intermediate host of either species may occasionally be exposed to infection by the appropriate host stage of the other (Rausch, 1995). Canids are infected from eating hydatid cysts in intermediate host viscera. The pathway to humans and intermediate hosts is the ingestion of eggs shed in the feces of infected canids. Domestic dogs become infected with *E. granulosis* from consuming the

viscera of hunter-killed cervids and with *E. multilocularis* from eating intermediate arvicoline rodent hosts (Castrodale, 2003).

Echinococcus granulosis is endemic throughout all of Alaska and Canada, whereas the range of *E. multilocularis* is roughly equivalent and limited to that of the arctic fox. The parasite is endemic in northern tundra regions, extending from western Alaska to Hudson Bay and to subarctic St. Lawrence Island and Nunavak Island. *Echinococcus multilocularis* is also found in some islands of the Canadian Arctic Archipelago, but has not been found on the northernmost islands of Canada (Eckert and Deplazes, 2004).

Human cases of cystic echinococcus (CE) caused by infection with *E. granulosis* have been reported throughout Alaska, except for the Aleutians. In contrast, human cases of alveolar echinococcus (AE) caused by *E. multilocularis* have only been reported on St. Lawrence Island and the North Slope (Castrodale, 2003). Since the 1950s, over 300 cases of echinococcosis have been reported to the Alaska State Section of Epidemiology, mostly from St. Lawrence Island or other rural parts of the State (Castrodale, 2003).

Historically, rates of echinococcosis increased when Inupiat Eskimos transitioned from nomadic to a sedentary lifestyle, as missionaries and the military established infrastructure. The sanitary effect of moving was lost and people began living in close proximity to dogs (and dog feces), which they relied on for transportation. Sled dogs had

access to infected caribou viscera discarded by hunters and/or infected microtine rodents. These conditions created hyperendemic foci, with the highest human infection rates ever recorded. On St. Lawrence Island, for example, the number of human AE cases reached 98/100,000 annually prior to the institution of education and control programs.

The most recent investigations of *Echinococcus* prevalence in Alaska were conducted on St. Lawrence Island between 1985 and 1990 to evaluate the effectiveness of *Echinococcus* control programs and near Barrow (western Alaska North Slope) during a snowy owl (*Nyctea scandiaca*) breeding ecology study in 2005 (Rausch et al., 1990; Holt et al., 2005). Rausch et al. (1990) found prevalence rates in voles (N=528) of 5% (95% CI: 3.4-7%[2]; an 83% reduction from pre-control measures) on St. Lawrence Island. Holt et al. (2005) found metacestode prevalence among brown lemmings (N=476) near Barrow, on Alaska's North Slope, to be lower, at 0.9% (95% CI: UL=LL[2]). Historical prevalence of AE (human infection with *E. multilocularis*) in the North Slope region, where host populations experience dramatic fluctuations, was also comparatively lower (Rausch and Fay, 2002). Rausch suggested higher prevalence in microtine intermediate and canid definitive hosts on St. Lawrence Island was facilitated by the relative stability of wild host populations (e.g. higher density).

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[2] Calculated here as described in: Hughes-Hanks JM, Rickard LG, Panuska C, Saucier JR, O'Hara TM, Dehn L, Rolland RM. 2005. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in five marine mammal species. Journal of Parasitology 91(5):1225-1228.

Since the 1990's, only eight cases of CE and no cases of AE have been reported to the Alaska State Section of Epidemiology (Castrodale, 2003). Nine suspected cases were reported to the Alaska State Section of Epidemiology between 2003 and 2010 (pers. comm., L. Castrodale 2010). A recent study prompted by the discovery of a cerebral cystic hydatid in a 6 year old girl in Saskatchewan, Canada demonstrated that at least 11% of humans in the village had antibodies to *E. granulosis* and eggs were detected (via PCR) in the feces of 6% of local dogs. Because dog ownership, hunting, and trapping were not detected as risk factors for specific antibody presence, Himsworth et al. (2010) hypothesized that exposure resulted from indirect contact with canine feces in the environment.

Although *Echinococcus* species have been well characterized in other regions of Alaska especially St. Lawrence Island, there is limited information available describing the parasite in its definitive hosts on the North Slope. Rausch (1967) documented *E. multilocularis* in 8.7% (95% CI: 5.2-13.4%[2]) of arctic fox (N=207) and 1 red fox (N=4) from the Arctic Coast (Colville Delta to Point Hope, including Brooks Range) based on parasite morphology alone. Furthermore, human cases of AE from the North Slope have not been evaluated beyond histopathology, and despite histopathological evidence of metacestodes of *E. multilocularis* in lemmings near Barrow (Holt et al., 2005), the parasite's identity was not confirmed.

Retreating sea ice, and other impacts of climate change, along with industrial encroachment and increasing human population size suggest that risk factors for parasitic infection may be changing on Alaska's North Slope, which presents a public health concern for Arctic residents. However, the distribution and abundance of *Echinococcus* species in this region is poorly known. Here, we use molecular genetic techniques to describe occurrence of *Echinococcus* in its definitive North Slope hosts. We also describe occurrence of two Taeniinae species in these same hosts. Furthermore, we suggest that canid definitive hosts, and particularly arctic fox, provide the best sentinels for monitoring change in *Echinococcus* infection pressure to humans due to their feeding ecology, distribution, shorter prepatentcy, and the availability of noninvasive and reliable means of parasite detection, and association with human habitation. In addition, the combined use of this sentinel and technology allow for monitoring which can assess risk of exposure (based on prevalence as well as strain identification) rather than relying on detection of end stage disease in humans.

4.3 METHODS

4.3.1 Sample Collection

We examined Arctic fox (N=46), sled dog (N = 12) and red fox (N = 1) fecal samples obtained from animals living along the Alaska North Slope. Samples were obtained opportunistically and thus do not constitute a random sample. The majority of samples were obtained in association with human populations and this facilitated our goal of assessing infection pressure to humans as this organism is of significant public health

concern. From a public health perspective, the determination of "true prevalence" was not the ultimate goal (and largely unfeasible). The evalation of presence versus absence of this parasite in animals in close associations with humans is of value in assessing health risk to humans.

Twenty-eight samples were collected by the North Slope Borough Veterinary Clinic and Department of Wildlife Management from foxes that were trapped for recreation and as part of the animal damage control (rabies) program for the Public Health Office. Samples were harvested between October 21 and December 7, 1999 from animals in the Barrow landfill (N=12), Barrow gravel pit (N=2), and near the DEW (Defense Early Warning)-line facility near Barrow (N=9) (Figure 4.1). Two arctic foxes were trapped in Kaktovik, 2 in Point Lay and the location of one harvested red fox was unknown (Figure 4.1). Samples were also derived from live arctic fox captured by University of Alaska Fairbanks personnel in June 2005 (N=8) and between June 30th and July 11th, 2007 (N=11) in the Prudhoe Bay Unit. Domestic canine samples were obtained from sled dogs belonging to one team from Barrow on February 25, 2007. Live captured arctic fox were taken using Tomahawk live traps baited with canned tuna throughout the Prudhoe Bay Unit approximately between 148°60' and 147°50' longitude and from the coast to approximately 70°00 (Figure 4.1). Capture of arctic fox and sampling procedures for live sampled arctic fox and sled dogs were approved by an independent animal care and use committee (University of Alaska Fairbanks, Institutional Animal Care and Use Committee Assurances 04-41, 05-15, and 07-30).

4.3.2 Sample Preparation and Molecular Analyses

All fecal (defecated) samples and fox colons were frozen at -80°C for at least two weeks prior to handling in order to reduce viability of *Echinococcus* spp. eggs. Approximately 3 gm of each fecal sample was suspended in 4.5 ml of 0.01 M phosphate-buffered saline, pH 7.2, containing 0.01 M EDTA (PBS-EDTA), and the suspension was filtered using sterile cheesecloth. The filtrate was centrifuged at 800 x g for 10 minutes and supernatant discarded; the pellet was washed two more times in PBS-EDTA at 1200 x g. After the final wash, the pellet was re-suspended in 1 ml of PBS-EDTA and used for DNA extraction with the FastDNA kit (MP Biomedicals, Solon, Ohio, USA) as previously described (da Silva et al., 1999), except for the substitution of binding matrix A for binding matrix F.Final purification used a QIAquick PCR purification kit (QIAGEN, Valencia, CA, USA).

After reviewing the published literature on genotyping techniques for *Echinococcus*, we chose primer sets targeting a gene located in the mitochondrial genome, NADH dehydrogenase-1 (ND1-F: 5-AGA TTC GTA AGG GGC CTA ATA-3; ND1-R: 5-ACC ACT AAC TAA TTC ACT TTC-3), which amplifies a 488bp fragment of the gene (Bowles 1992). We selected primer sequences, which were conserved among all *Echinococcus* species and subtypes identified to date (*Echinococcus multilocularis, Echinococcus granulosis* [G1-G10], *Echinococcus shiquicus*), yet which would amplify products containing enough diversity in order to differentiate between species and subtypes. PCR amplification was performed in 50µl reactions containing 5µl DNA,

200μm of each dNTP, 2.5 mM $MgCl_2$, 0.2 μm of each primer and 1.5 U of AmpliTaq Gold. The PCR was performed with an initial denaturation step of 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 30s, 48 °C for 60s and 72 °C for 60s. All samples in which an amplicon of the appropriate size was present were run in duplicate. Amplicons were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. Direct sequencing was performed in both the forward and reverse directions using the same primers used in the PCR with use of the BigDye™ Terminator Kit (Applied Biosystems, Foster City, CA, USA) and an automated sequencer (ABI 3130 Genetic Analyzer, Applied Biosystems) according to the manufacturer's instructions. Primer sequences were removed prior to sequence analyses. The complete sequence assemblies were created using Lasergene® v7.0 (DNASTAR, Inc., Madison, WI, USA) using nucleotide data with quality higher than 20 (e.g. the base call is at least 99% accurate; Ewing and Green, 1998). Sequences resulting from a BLAST search of the National Center for Biotechnology Information (NCBI) database were imported and aligned with fox sequences using Clustal W in the Lasergene® v7.0 software package. Appropriate likelihood models were determined using the Akaike Information Criterion implemented in ModelTest 3.7, PAUP* 4.0b10 (Posada and Crandall, 1998; Posada and Buckley, 2004). PHYML (Guindon and Gascuel, 2003) was used to construct maximum likelihood (ML) trees for partial nad1 gene sequences. Trees were generated for each of the three species of parasite found in order to assess their relatedness to isolates of the same species representative of a given region, as available in GenBank.

4.4 RESULTS

The primers used in this study amplified *Echinococcus* and Taeniinae mitochondrial DNA from 95.7% and 100% of arctic fox samples and sled dog samples. To determine prevalence of *Echinococcus,* we examined the PCR products sequences. *Echinococcus multilocularis* were found only in arctic fox collected in 1999 in Barrow near the DEW-line, landfill or gravel pit. *Taenia crassiceps* was detected in animals sampled in all three years, at all locations, including both arctic fox and sled dogs in Barrow. *Taenia polyacantha* was detected in one arctic fox in 1999 (Table 4.1a). Only *T. crassiceps* was detected in sled dogs and although PCR products of expected size were generated from all 12 animals, sequences could not be resolved for 7 dogs sampled (Table 4.1b) due to the presence of multiple peaks. Isolates of a given species of parasite from individual arctic and red fox, as well as sled dogs, were identical.

Based on Clustal W and maximum likelihood analysis, the Alaska arctic fox isolate of *Echinococcus* was most closely related to an isolate of *E. multilocularis* from China (2 nucleotide differences, Figure 4.2) and least related to the European *E. multilocularis* isolate (Figures 4.3). This finding is consistent with studies of *Echinococcus* in other Alaskan species using other genes. Interestingly, the only 2 positions where the Alaska and China isolates differed were identical in all isolates except the Alaska fox isolate (Figure 4.2).

The *T. crassiceps* North Slope, Alaska arctic fox isolate contained one polymorphism (G/C) at position 254 (Figure 4.4). This was the only difference from identical isolates from a Svalbard arctic fox and Svalbard southern vole (*Microtus levis*), sequences with which it formed a clade (Figure 4.5). The North Slope isolate was more closely related to isolates from Svalbard than to an isolate from a meadow vole (*Microtus pennsylvanicus*) in Fairbanks (Interior Alaska, 5 nucleotide differences), based on maximum likelihood analysis (Figures 4.4 and 4.5). In general, there was consistency in which nucleotide sites were variable across isolates from various regions.

The *T. polyacantha* North Slope, Alaska arctic fox isolate demonstrated the greatest homology with a sequence isolated from an arctic fox sampled in Svalbard and from a brown lemming in Canada (Figure 4.6). However, it did not form a clade with any sequence (Figure 4.7) in the analysis. The Alaska isolate differed from both by only two nucleotides (Figure 4.6). The Svalbard and Canada sequences both had a T at position 80, whereas the Alaska sequence contained a G. The Alaska arctic fox isolate differed from both the Svalbard isolate and Canada lemming isolate, each by an additional transition (different nucleotide for each isolate, Figure 4.4).

4.5 DISCUSSION

Our examination of the mitochondrial nad1 gene fragment confirmed, for the first time, the identity of *E. multilocularis* in arctic fox, the definitive host for the sylvatic cycle of this organism, from Alaska's North Slope. Previous examination of *Echinococcus* in this

region tentatively identified the species as *E. multilocularis*, however the parasite's identity at the molecular level was not investigated. Rausch identified *E. multilocularis* in arctic and red fox in this region based on morphology of organisms observed in arctic and red fox (Rausch, 1967). Furthermore, human cases of AE from the North Slope had not been evaluated beyond histopathology, and despite histopathological evidence of metacestodes of *E. multilocularis* (Holt et al., 2005), the parasite's identity was not confirmed at the genetic level or at the molecular level.

The primers used in this study to amplify *Echinococcus* mitochondrial DNA were not specific to *Echinococcus*, but also amplified other Taeniinae present in fecal samples. As a result, PCR products were obtained from 95.7% and 100% of arctic fox samples and sled dog samples, respectively, and 1 red fox sample. In order to determine the true prevalence of *Echinococcus,* therefore we needed to examine the sequences of the resulting PCR products. The most common Taeniidae observed was *Taenia crassiceps* (N=22), followed by *E. multilocularis* (N=7) and *Taenia polyacantha* (N=1). Both organisms have life cycles similar to that of *E. multilocularis* and they are acquired by arctic fox through the consumption of arvicoline rodents that serve as intermediate hosts. There were seven amplification products sequenced from sled dogs that could not be unequivocally identified. Based on the electropherogram, it was apparent that multiple sequences were present, indicating the presence of at least 2 different species. Because these primers amplify multiple species of Taeniidae, cloning of amplification products would be necessary for the resolution of these sequences.

4.5.1 Phylogenetics

The genus *Taenia* contains greater genetic diversity than *Echinococcus.* Among *Taenia* species, there is the greatest intraspecifc variability among *T. polyacantha* isolates (0-9.2% based on nad1 gene) and the least among *T. crassiceps* (0-1.6% nad1 gene) (Lavikainen et al., 2008). The closer genetic relationship of the North Slope arctic fox isolates with southern voles from Svalbard than with isolates from meadow voles in Fairbanks, Alaska may reflect differences in ecoregion. Both Svalbard and the North Slope are in the Arctic zone, whereas Fairbanks, Alaska is Subarctic. The *T. polyacantha* Alaska isolate clustered among the arctic strains (Lavikainen et al., 2008).

Echinococcus multilocularis consists of three clades based on geographic origin: Europe, Asia, and North America. Previous genetic (cob, nad2, cox1 and elp exons) studies of *E. multilocularis* on isolates from St. Lawrence Island Alaska have found both North American and Asian strains present (Nakao et al., 2009). The authors of this phylogeographic analysis suggested the observed coexistence may have resulted from an evolutionary scenario in which distinct parasite populations derived from glacial refugia have been maintained by indigenous host mammals. Throughout periods of the Pleistocene, sea levels fell as a result of glaciation forming the Bering land bridge, which connected Asia and North America (Pielou, 1991).

We could not assess the relatedness of our isolate to those representing North America since these studies did not examine the nad 1 gene. However, based on the maximum

likelihood analysis of analysis of strains for which genetic information was available, the Alaska strain is most closely related to a strain from China (Nakao et al., 2009). This study provides the first evidence of the Asian strain not only on St. Lawrence Island, but on the mainland of Alaska. This is not unexpected since fox are known to travel great distances, including between islands and mainland. Such travel has been implicated in the spread of rabies and distemper epizootics (Ritter, 1981; Bohm et al., 1989; Campbell et al., 2007) and thus could easily disperse the parasite.

Echinococcus multilocularis has apparently undergone range expansion in European over the last few decades, generating increasing awareness of this parasite as a public health issue. Strains previously thought to exist only in Asia were later documented in Eastern European nations including Poland and Estonia (Moks et al., 2005). These findings are supported in the phylogenetic analyses here, which demonstrates these Eastern European strains to be more closely related to the Chinese strain than the European isolate. Due to the lack of previous studies in this area however, it is uncertain if this observation is a actually a result of recent range expansion or if the parasite was already endemic to the area.

4.5.2 Concerns for Climate Change Impacts on Host Ecology

On the North Slope of Alaska the brown lemming, collared lemming (*Dicrostonyx groenlandicus*), tundra vole, and singing vole (*Microtus miurus*) are present (MacDonald and Cook, 2009) and therefore represent potential intermediate hosts for *E.*

multilocularis. The arctic and red fox are definitive hosts in the sylvatic cycle of the parasite in this region. The lemming population undergoes high amplitude fluctuations every 3-4 years and can comprise up to 90% of the diet of arctic fox in peak years (Rausch and Fay, 2002). The arctic fox population in northern Alaska follows the cyclic abundance of lemmings and other rodents. Populations flourish when these prey are abundant and decline when prey populations decline, due to food availability. Arctic fox can use sea ice extensively for forage (e.g. seal carrion) presumably more so in years when winter foods are limited, for example in low lemming years. Pamperin et al. (2008) followed individuals outfitted with location transmitters and suggested that diets for arctic fox using sea-ice were likely 100% marine while on the sea-ice given their consistent locations at distances that would preclude periodic trips to shore to feed on terrestrial resources. Rabies outbreaks occur in Alaska arctic fox populations during the winter months (Ritter, 1981) when rodent availability is lowest and access to birds (and eggs) as alternative prey is lacking, approximately every 3-5 years when rodent populations drop. It is hypothesized that traveling over long distances, congregation at food sources and scavenging on infected carcasses in combination with nutritional stress may facilitate such outbreaks. There is concern that diminishing sea ice may lead to increased presence of foxes and polar bears (*Ursus maritimus*), for example at human settlements (e.g. landfills and whale carcasses remains) and industrial sites where anthropogenic food sources are present. For density dependent diseases, the presence of anthropogenic food sources such as baiting and waste stations that concentrate animals in a given area can greatly enhance transmission rates (Wobeser, 2006). Although *E.*

multilocularis is less density dependent than for example canine distemper virus (morbillivirus), owing to the fact that it is maintained in multiple hosts and therefore not tied to a critical community size for one population, such sites of attraction could create transmission foci.

The North Slope is currently a region of apparent relative low endemicity for *E. multilocularis*, however this may change as alterations in climate cause range changes of suitable intermediate and definitive hosts, and with the encroachment of industry (areas of definitive host concentration). It is recognized that the Arctic is especially vulnerable to climate change because global warming is most pronounced at high latitudes. The arctic tundra is predicted to shrink with global warming as the boreal forest zone moves north (ACIA, 2004). Although this shift is not of immediate imminence, ecological processes and species inhabiting the tundra ecosystem are likely to be affected well before the tundra becomes forested (Fuglei and Ims, 2008). With warming, population cycles of lemmings and voles are likely to decrease in amplitude and become increasingly unstable as such cycles appear to depend on long, cold and stable winters (Yoccoz and Ims, 1999; Aars and Ims, 2002). This is because warmer, unstable winters with repeated freeze-thaw events can result in the formation of ice crust, reducing access to forage. However, in the short term arctic fox populations could benefit from the increased availability of carrion following population crashes in herbivores resulting from the same phenomenon. Such events have been documented in reindeer and musk oxen populations in different locations in the Arctic (Aanes et al., 2000; Forchhammer

and Boertmann, 1993).

The recent northward expansion of the red fox, a dominant competitor and predator of arctic fox, is well documented (Tannerfeldt et al., 2002; Hersteinsson and MacDonald, 1992). Observations suggest the northward expansion of red fox has resulted in direct mortality of arctic fox by the larger, more aggressive red fox (Pamperin et al., 2006). The availability of anthropogenic food sources at village landfills and around industrial sites can result in conspecific and interspecific aggregations of animals, potentially enhancing parasite transmission. Furthermore, data from collared animals revealed that arctic fox living near the Prudhoe Bay Unit (actively leased and utilized oil and gas field located on the North Slope) remained in the local area throughout winter (Pamperin, 2008). In contrast, arctic fox living in the National Petroleum Reserve-Alaska (NPR-A), an undeveloped area, traveled greater distances and spent considerable time scavenging carrion on the sea-ice during the winter (Pamperin, 2008; Pamperin et al., 2008).

The reduction of the sea ice has implications for the ability of arctic fox to access marine resources. Species which depend upon the sea-ice as a platform for resting, breeding, and hunting such as walrus (*Odobenus rosmarus*), some seals and polar bear are anticipated to be significantly affected by the loss of sea ice (Burek et al., 2008; Wiig et al., 2008). Similarly, the ability of arctic fox to hunt ringed seal (*Pusa hispida*) pups and scavenge on the remains of polar bear kills is likely to be diminished. Such alterations in arctic fox feeding ecology have significant implications for the transmission dynamics

of the Taeniidae since they have a trophic dependent life cycle. As the diet of the arctic fox changes, so will its parasite fauna.

4.5.3 Concern for Climate Change Impacts on Echinococcosis and Human Health

Public health officials are concerned that climate change may increase *Echinococcus* exposure of humans in the Arctic (Parkinson and Butler, 2005). We suggest that canid definitive hosts, and particularly arctic fox, present the best sentinels for monitoring change in parasite prevalence due to their feeding ecology, shorter prepatency (e.g. relative to humans) and the availability of noninvasive and reliable means of parasite detection. The arctic fox presents a particularly useful sentinel due to its ubiquity in areas of endemicity and because it is sometimes targeted in control programs to reduce the public health threat of rabies and in recreational use for furs. Thus, *Echinococcus* can be monitored opportunistically during the course of these efforts, in a similar manner as our work presented here.

Change in infection pressure of *Echinococcus* to humans and assessment of change in human infection rates will be difficult to detect due to the long duration between infection and onset of symptoms. Humans are believed to be infected at a young age, with preclinical periods of 20 to 30 years (Rausch et al., 1990). Growth of *Echinococcus* cysts is variable, with rates as slow as slow as 1 mm to 5 mm per year (Palowski et al., 2001). An infected individual may remain asymptomatic for decades or permanently, depending upon whether cyst's location induces pathologic changes in the infected

organ and/or if the cyst ruptures. Furthermore, diagnostics including imaging techniques, mainly ultrasound and computed tomography examination for abdominal echinococcosis and X-ray for lung echinococcosis, and immunodiagnostic tests, are costly and sometimes inaccurate (Eckert et al., 2001). Species confirmation of causative agent via PCR is not routinely performed, further limiting assessment of infection patterns by epidemiologists. The ultrasound examination can be used under field conditions for population screening, but the others require a clinical setting. In rural Alaska, where most infections occur, diagnosis can be limited by access to such technologies and expert medical professionals. Perhaps most importantly, monitoring the end stage disease in humans does not adequately address potential and actual exposure public health concerns.

Once infected canids are identified in a defined area, measures to prevent infection in humans can be undertaken. Control measures such as the administration of praziquantel (a parasiticide) and changes in dog husbandry practices conbined with education are demonstrated means of reducing human infection with *Echinococcus* (Rausch et al., 1990). The prophylactic administration of partasiticide is costly and inefficient due to the window of time in which canids can be re-infected (Eckert et al., 2001).

The prepatent period in canids is typically around 42 and 28 days for *E. granulosis* and *E. multilocularis*, respectively (Eckert et al., 2001). Thus, changes in parasite levels can be detected on a timescale that more closely follows alterations in the ecology of the

disease as they occur in real time. Eggs are shed in the feces of canid definitive hosts therefore necropsy is not required for diagnosis. Detection of metacestodes in sylvatic intermediate hosts (rodents and cervids), however, does require necropsy. Furthermore, the development *E. granulosis* metacestodes in cervids is slower in these long-lived species. The prevalence in intermediate hosts is lower than in canid definitive hosts. As a result, sampling of many more animals is required in order to establish prevalence rates with narrow confidence intervals, and to detect change. The use of PCR can reliably distinguish between species and strains of *Echinococcus* and the application of phylogenetics allows for the evaluation of differences. This distinction has particular importance in public health since strains of *Echinococcus* spp. can be of varying virulence (Rausch et al., 1990; Moro and Schantz, 2006). Such measures cannot be accomplished using histopathology alone. Knowledge of strain types that are currently present will allow for observation of alterations in parasite ecology. The use of molecular technology for monitoring *Echinococcus* in canids offers the most rapid, economical and detailed epidemiological insights into changes in the ecology of the disease, when compared to following infection in humans or intermediate hosts.

4.6 CONCLUSION

We have unequivocally identified the species of *Echinococcus* present in arctic fox on the North Slope of Alaska as *E. multilocularis*. There is, however, little information available regarding the current ecology and transmission dynamics of *E. multilocularis* in the High Arctic. With anticipated changes in Arctic ecology due to climate change

and increasing anthropogenic influences, prevalence of this zoonotic agent is likely to change with shifts in host ranges. Because *Echinococcus* infection poses a significant public health concern; understanding its ecology in the Arctic and monitoring its prevalence will be necessary to understand the human health consequences of ongoing and projected changes in the Arctic. We suggest that canids present the best sentinels for monitoring the impact of change on the ecology of *Echinococcus*. Such studies can be conducted opportunistically in cooperation with rabies and predator control programs, trapping and health assessments in targeted areas. Although we could not assess the relationship of the strain we isolated from arctic fox to North America strains (genetic information was unavailable in the database), the North Slope isolate was most similar to Asian isolates based on maximum likelihood analysis. This observation is consistent with the previous detection of Asian isolates in St. Lawrence Island, Alaska. Sequence analysis of isolates of *T. crassiceps* and *T. polyacantha* demonstrated the greatest homology with isolates derived from the same host (*V. lagopus*).

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4.8 REFERENCES

Aanes R, Saether BE, Oritsland NA. 2000. Fluctuations of an introduced population of Svalbard reindeer: the effect of density dependence and climatic variation. Ecography 23(4):437-443.

Aars J, Ims RA. 2002. Climatic and intrinsic determinants of population demography: the winter dynamics of tundra vole populations. Ecology 83(12):3449-3456.

ACIA. 2004. Impacts of a warming Arctic. Arctic Climate Impact Assessment. Cambridge, United Kingdom: Cambridge University Press, 144 pp.

Bohm J, Blixenkrone-Møller M, Lund E. 1989. A serious outbreak of canine distemper among sled-dogs in northern Greenland. Arctic Medical Research 48(4):195-203.

Bradley MJ, Kutz SJ, Jenkins E, O'Hara TM. 2005. The potential impact of climate change on infectious diseases of arctic fauna. International Journal of Circumpolar Health 64(5):468-477.

Burek KA, Gulland FMD, O'Hara TM. 2008. Effects of climate change on Arctic marine mammal health. Ecological Applications 18(2) Supplement:S126-134.

Campbell D, Welch K, Baker C, Overell J. 2007. Canine distemper virus in sled dogs-Nunavut and NT. Canadian Cooperative Wildlife Health Centre Newsletter 13(1):8-9.

Castrodale L. 2003. Echinococcosis in Alaska. State of Alaska Epidemiology Bulletin No. 2, February 10, 2003.

da Silva AJ, Bornay-Llinares FJ, Moura INS, Slemenda SB, Tuttle JL, Pieniazek NJ. 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Molecular Diagnostics 4(1):1-8.

Dinkel A, von Nickisch-Rosenegk M, Bilger B, Merli M, Lucius R, Romig T. 1998. Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis as an alternative to necropsy. Journal of Clinical Microbiology 36(7):1871-1876.

Eckert J, Deplazes P. 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clinical Microbiology Reviews 17(1):107–135.

Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowlers M. 2001. Echinococcosis in animals: clinical aspects, diagnosis and treatment. In: WHO/OIE Manual on Echinococcosis in humans and animals: a public health problem of global concern, Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (editors), Paris: World Organisation for Animal Health, pp 73-100.

Ewing B, Green P. 1998. Basecalling of automated sequencer tracers using Phred II. Genome Research 8(3):186-194.

Forchhammer M, Boertmann D. 1993. The muskoxen *Ovibos moschatus* in north and northeast Greenland: Population trends and influence of abiotic parameters on population dynamics. Ecography 16(4):299 -308.

Fuglei E, Ims RA. 2008. Global warming and effects on the arctic fox. Science Progress 91(2):175-191.

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5):696-704.

Jenkins DJ, Romig T, Thompson RC. Emergence/re-emergence of *Echinococcus* spp.-a global update. International Journal for Parasitology 35(11-12):1205-1219.

Hersteinsson P, MacDonald DW.1992. Interspecific competition and the geographical distribution of red and arctic foxes, *Vulpes vulpes* and *Alopex lagopus*. Oikos 64(3):505-515.

Himsworth CG, Jenkins E, Hill JE, Nsungu M, Ndao M, Andrew Thompson RC, Covacin C, Ash A, Wagner BA, McConnell A, Leighton FA, Skinner S. 2010. Emergence of sylvatic *Echinococcus granulosus* as a parasitic zoonosis of public health concern in an indigenous community in Canada. American Journal of Medicine and Tropical Hygiene 82(4):643-645.

Hoberg EP. 2002. *Taenia* tapeworms: their biology, evolution and socioeconomic significance. Microbes and Infection 4(8):859–866.

Holt DW, Hanns C, O'Hara TM, Burek K, Frantz R. 2005. New distribution records of *Echinococcus multilocularis* in the brown lemming from Barrow, Alaska. Journal of Wildlife Disease 41(1):257-259.

Hughes-Hanks JM, Rickard LG, Panuska C, Saucier JR, O'Hara TM, Dehn L, Rolland RM. 2005. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in five marine mammal species. Journal of Parasitology 91(5):1225-1228.

Lavikainen A, Haukisalmi V, Lehtinen MJ, Henttonen H, Oksanen A, Meri S. 2008. A phylogeny of members of the family Taeniidae based on the mitochondrial cox1 and nad1 gene data. Parasitology 135(12):1457-1467.

MacDonald SO, Cook JA. 2009. Recent Mammals of Alaska, Chicago, IL: The University of Chicago Press.

Moks E, Saarma U, Valdmann H. 2005. *Echinococcus multilocularis* in Estonia. Emerging Infectious Diseases 11(12):1973-1974.

Moro P, Schantz PM. 2006. Cystic echinococcosis in the Americas. Parasitology International 55(S1):181-186.

Nakao M, Xiao N, Okamoto M, Yanagida T, Sako Y, Ito A. 2009. Geographic pattern of genetic variation in the fox tapeworm *Echinococcus multilocularis*. Parasitology International 58(4):384–389.

Obwaller A, Schneider R, Walochnik J, Gollackner B, Deutz A, Janitscheke K, Aspock H, Auer H. 2004. *Echinococcus granulosus* strain differentiation based on sequence heterogeneity in mitochondrial genes of ctyochrome c oxidase-1 and NADH dehydrogenase-1. Parasitology 128(Pt 5):569-575.

Pawlowski ZS, Eckert J, Vuitton DA, Ammann RW, Kern P, Craig PS, Dar KF, De Rosa F, Filice C, Gottstein B, Grimm F, Macpherson CNL, Sato N, Todorov T, Uchino J, von Sinner W, Wen H. 2001. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern, Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (editors), Paris, France: World Organisation for Animal Health, pp 21-72.

Pamperin NJ, Follmann EH, Petersen B. 2006. Interspecific killing of an arctic fox by a red fox at Prudhoe Bay, Alaska. Arctic 59(4):361-364.

Pamperin, NJ. 2008. Winter movements of arctic foxes in northern Alaska measured by satellite telemetry. Master's Thesis, University of Alaska Fairbanks.

Pamperin NJ, Follmann EH, Person BT. 2008. Sea-ice use by arctic foxes in northern Alaska. Polar Biology 31(11):1432-2056.

Parkinson AJ, Butler JC. 2005. Potential impacts of climate change on infectious diseases in the Arctic. International Journal of Circumpolar Health 64 (5):478-486.

Pielou EC. 1991. After the Ice Age: the return of life to glaciated North America. Chicago: University of Chicago Press.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9):817-818.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Systematic Biology 53(5):793-808.

Rausch RL. 1967. On the ecology and distribution of *Echinococcus spp.* (*Cestoda*: Taeniidae), and characteristics of their development in the intermediate host. Annales de Parasitologie (Paris) 42(1):19-63.

Rausch RL. 1995. Life cycle patterns and geographic distribution of *Echinococcus* species. In: *Echinococcus* and hydatid disease, Thompson RCA, Lymbert AL (editors), Oxfordshire, UK: CAB International, pp 89–134.

Rausch RL. 2003. Cystic echinococcosis in the Arctic and Sub-Arctic. Parasitology 127 Supplement:S73-85.

Rausch RL, Fay FH. 1988. Postoncopheral development and cycle of *Taenia polyacantha* Leuckart, 1856 (Cestoda: Taeniidae). Annales de Parasitologie Humaine et Comparee 63(4):263–277 and 334–348.

Rausch R, Wilson JF, Schantz PM. 1990. A program to reduce the risk of infection by *Echinococcus multilocularis*: The use of praziquantel to control the cestode in a village in the hyperendemic region of Alaska. Annals of Tropical Medicine and Parasitology 84(3):239–250.

Rausch RL, Fay FH. 2002. Epidemiology of alveolar echinococcosis with reference to St. Lawrence Island, Bering Sea, In: Cestode Zoonoses: Echinococcus and Cysticercosis—An emergent and global problem, Craig P, Z Pawlowski ZS (editors), Amsterdam, The Netherlands: IOS Press, pp 309-325.

Ritter D. 1981. Rabies. In: Alaskan Wildlife Diseases, (editor) Dieterich RA. Fairbanks, Alaska: University of Alaska, pp 6-12.

Romig T, Thoma D, Weible AK. 2006. *Echinococcus multilocularis*--a zoonosis of anthropogenic environments? Journal of Helminthology 80(2):207-212.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds AJ. 2003. Fingerprints of global warning on wild animals and plants. Nature 421(6918):57-60.

Stehr-Green JK, Stehr-Green PA, Schantz PM, Wilson JF, Lanier A. 1988. Risk factors for infection with *Echinococcus multilocularis* in Alaska. American Journal of Tropical Medicine and Hygiene 38(2):380-385.

Tannerfeldt M, Elmhagen B, Angerbjorn A. 2002. Exclusion by interference competition? The relationship between red and arctic foxes. Oecologia 132(2):213-220.

Wiig Ø, Aars J, Born EW. 2008. Effects of climate change on polar bears. Science Progress 91(2):151-173.

Wilson JF, Rausch RL, Wilson FR. 1995. Alveolar hydatid disease: review of the surgical experience in 42 cases of active disease among Alaskan Eskimos. Annals of Surgery 222(3):315-323.

Wobeser GA. 2006. Essentials of Disease in Wild Animals, Ames, IA: Wiley-Blackwell Publishing.

Yoccoz NG, Ims RA.1999. Demography of small mammals in cold regions: The importance of environmental variability. Ecological Bulletins 47(1):137-144.

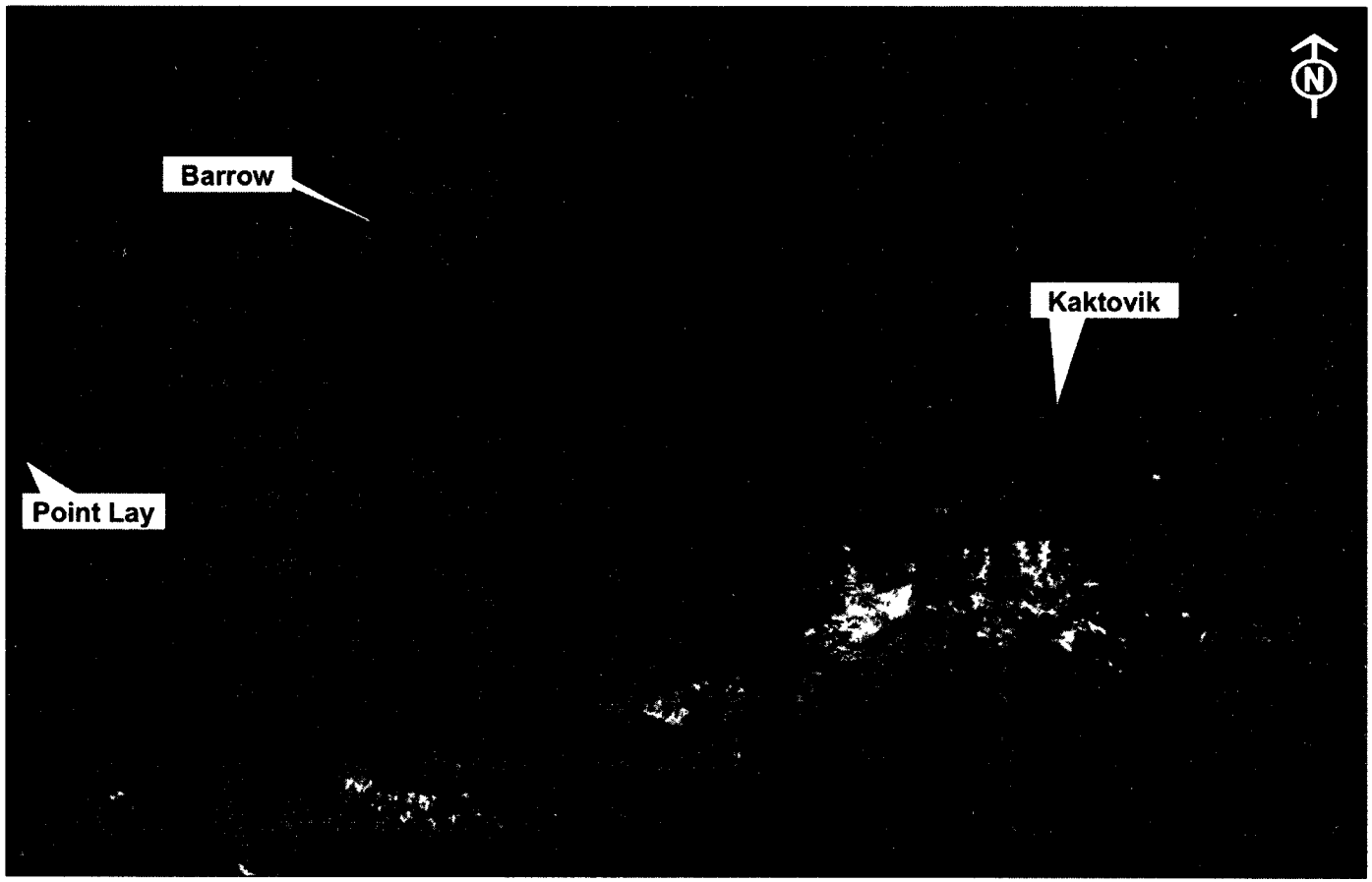


Figure 4.1 Map of the Alaska North Slope displaying fox and dog sampling area.

```
E.multilocularis_Ak-V.lagopus*      AGGTTGGTTTTGCTGGTTTGTTGCAGAGATTTGCTGATTTGTTAAAGTTA 50
E.multilocularis_Ch-Human           AGGTTGGTTTTGCTGGTTTGTTGCAGAGATTTGCTGATTTGTTAAAGTTA 50
E.multilocularis_Gr-Rodent          ----------GCTGGTTTGTTGCAGAGATTTGCTGATTTGTTAAAGTTA 39
E.multilocularis_Po-V.vulpes_7      --GTTS-CCTTGCTGGTTT-TTGCAGAGATTTGCTGATTTGTTAAAGTTA 46
E.multilocularis_Es-V.vulpes        ------------------------------GCTGATTTGTTAAAGTTA 18
E.multilocularis_Po-V.vulpes_7      ---------TTGCTGGTTN-YTGCAGAGATTTGCTGATTTGTTAAAGTTA 40
                                                                  ******************

E.multilocularis_Ak-V.lagopus*      GTGATCAAGTTTAAGAATTTTTATTTTCAAAGTCGTAGGTATATTGGTTT 100
E.multilocularis_Ch-Human           GTGATCAAGTTTAAGAATTTTTATTTTCAAAGTCGTAGGTATATTGGTTT 100
E.multilocularis_Gr-Rodent          GTGATCAAGTTTAAGAATTTTTATTTTCAAAGTCGTAGGTATATTGGTTT 89
E.multilocularis_Po-V.vulpes_7      GTGATCAAGTTTAAGAATTTTTATTTTCAAAGTCGTAGGTATATTGGTTT 96
E.multilocularis_Es-V.vulpes        GTGATCAAGTTTAAGAATTTTTATTTTCAAAGTCGTAGGTATATTGGTTT 68
E.multilocularis_Po-V.vulpes_7      GTGATCAAGTTTAAGAATTTTTATTTTCAAAGTCGTAGGTATATTGGTTT 90
                                    **************************************************

E.multilocularis_Ak-V.lagopus*      GTTGGGTGTTTTTTTTGTTAATAATTTTGGTTATTATATATTCTTTTATTT 150
E.multilocularis_Ch-Human           GTTGGGTGTTTTTTTTGTTAATAATTTTGGTTATTATATATTCTTTTATTT 150
E.multilocularis_Gr-Rodent          GTTGGGCGTTTTTTTTGTTAATAATTTTGGTTATTATATATTCTTTTATTT 139
E.multilocularis_Po-V.vulpes_7      GTTGGGTGTTTTTTTTGTTAATAATTTTGGTTATTATATATTCTTTTATTT 146
E.multilocularis_Es-V.vulpes        GTTGGGTGTTTTTTTTGTTAATAATTTTGGTTATTATATATTCTTTTATTT 118
E.multilocularis_Po-V.vulpes_7      GTTGGGTGTTTTTTTTGTTAATAATTTTGGTTATTATATATTCTTTTATTT 140
                                    ****** ****************************************

E.multilocularis_Ak-V.lagopus*      ATGGTAGATATTATAGTGTTAGTTATAATAGTCTTTCAGTATTGTGGTTT 200
E.multilocularis_Ch-Human           ATGGTAGATATTATAGTGTTAGTTATAATAGTCTTTCAGTATTGTGGTTT 200
E.multilocularis_Gr-Rodent          ATGGTAGATATTATAGTGTTAGTTATAATAGTCTTTCAGTATTGTGGTTT 189
E.multilocularis_Po-V.vulpes_7      ATGGTAGATATTATAGTGTTAGTTATAATAGTCTTTCAGTATTGTGGTTT 196
E.multilocularis_Es-V.vulpes        ATGGTAGATATTATAGTGTTAGTTATAATAGTCTTTCAGTATTGTGGTTT 168
E.multilocularis_Po-V.vulpes_7      ATGGTAGATATTATAGTGTTAGTTATAATAGTCTTTCAGTATTGTGGTTT 190
                                    **************************************************

E.multilocularis_Ak-V.lagopus*      TTAGCTGTTGCTAGTATTTCTAGGTATTCTTTGTTGTGTGCTGGTTGGGG 250
E.multilocularis_Ch-Human           TTAGCTGTTGCTAGTATTTCTAGGTATTCTTTGTTGTGTGCTGGTTGGGG 250
E.multilocularis_Gr-Rodent          TTAGCTGTTGCTAGTATTTCTAGGTATTCTTTGTTGTGTGTTGGTTGGGG 239
E.multilocularis_Po-V.vulpes_7      TTAGCTGTTGCTAGTATTTCTAGGTATTCTTTGTTGTGTGCTGGTTGGGG 246
E.multilocularis_Es-V.vulpes        TTAGCTGTTGCTAGTATTTCTAGGTATTCTTTGTTGTGTGCTGGTTGGGG 218
E.multilocularis_Po-V.vulpes_7      TTAGCTGTTGCTAGTATTTCTAGGTATTCTTTGTTGTGTGCTGGTTGGGG 240
                                    ************************************* *********
```

Figure 4.2 Clustal W alignment for partial nad1 gene sequence of *E. multilocularis* isolated from arctic foxes in 1999, northern Alaska.

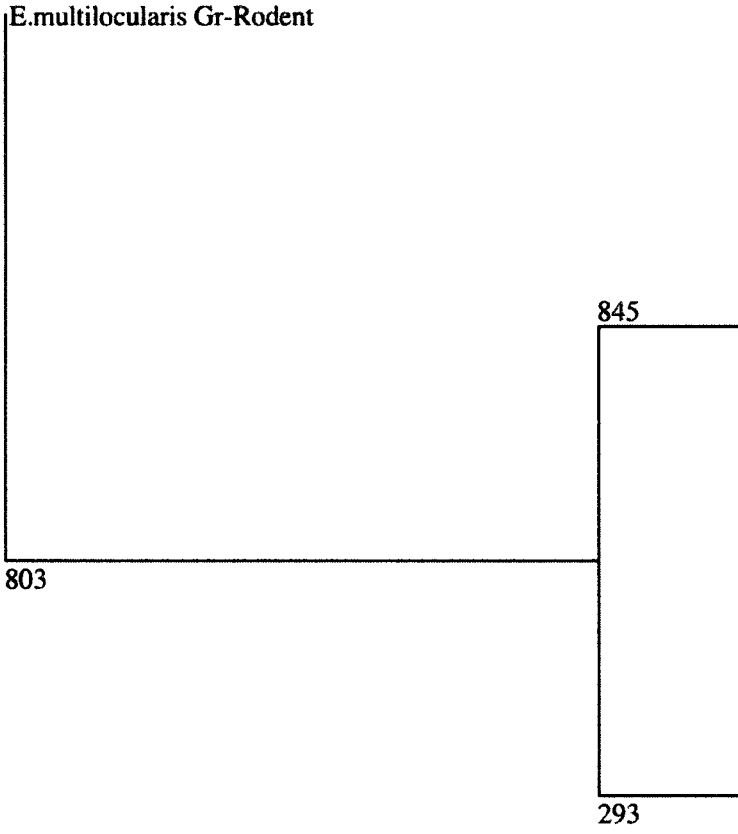
```
E.multilocularis_Ak-V.lagopus*      TAGTTACAATAAATATTCGTTTTTAAGTTCTGTTCGATGTGCTTTTGGGT 300
E.multilocularis_Ch-Human           TAGTTACAATAAATATTCGTTTTTAAGTTCTGTTCGATGTGCTTTTGGGT 300
E.multilocularis_Gr-Rodent          TAGTTACAATAAATATTCGTTTTTAAGTTCTGTTCGATGTGCTTTTGGGT 289
E.multilocularis_Po-V.vulpes_7      TAGTTACAATAAATATTCGTTTTTAAGTTCTGTTCGATGTGCTTTTGGGT 296
E.multilocularis_Es-V.vulpes        TAGTTACAATAAATATTCGTTTTTAAGTTCTGTTCGATGTGCTTTTGGGT 268
E.multilocularis_Po-V.vulpes_7      TAGTTACAATAAATATTCGTTTTTAAGTTCTGTTCGATGTGCTTTTGGGT 290
                                    **************************************************

E.multilocularis_Ak-V.lagopus*      CTGTTAGGTTTGAAGCTTGTTTTATGTGTGTGGTAATTTTTTGTTCTTTG 350
E.multilocularis_Ch-Human           CTGTTAGGTTTGAAGCTTGTTTTATGTGTGTGGTAATTTTTTGTTCTTTG 350
E.multilocularis_Gr-Rodent          CTGTTAGGTTTGAAGCTTGTTTTATGTGTGTGGTAATTTTTTGTTCTTTG 339
E.multilocularis_Po-V.vulpes_7      CTGTTAGGTTTGAAGCTTGTTTTATGTGTGTGGTAATTTTTTGTTCTTTG 346
E.multilocularis_Es-V.vulpes        CTGTTAGGTTTGAAGCTTGTTTTATGTGTGTGGTAATTTTTTGTTCTTTG 318
E.multilocularis_Po-V.vulpes_7      CTGTTAGGTTTGAAGCTTGTTTTATGTGTGTGGTAATTTTTTGTTCTTTG 340
                                    **************************************************

E.multilocularis_Ak-V.lagopus*      TGTTACTGTAGGTATAATTTGATTGATTTTTATTATAGTTGTTGATGAGG 400
E.multilocularis_Ch-Human           TGTTACTGTAGGTATAATTTGATTGATTTTTATTATAGTTGTTGATGAAG 400
E.multilocularis_Gr-Rodent          TGTTACTGTAGGTATAATTTGGTTGATTTTTATTATAGTTGTTGATGAAG 389
E.multilocularis_Po-V.vulpes_7      TGTTACTGTAGGTATAATTTGATTGATTTTTATTATAGTTGTTGATGAAG 396
E.multilocularis_Es-V.vulpes        TGTTACTGTAGGTATAATTTGATTGATTTTTATTATAGTTGTTGATGAAG 368
E.multilocularis_Po-V.vulpes_7      TGTTACTGTAGGTATAATTTGATTGATTTTTATTATAGTTGTTGATGAAG 390
                                    ********************.**************************.*
E.multilocularis_Ak-V.lagopus*      TTTGTTGTTGTTTCCATTGATTTATGGATTGTTTTTGGTGTGTGTGCTAT 450
E.multilocularis_Ch-Human           CTTGTTGTTGTTTCCATTGATTTATGGATTGTTTTTGGTGTGTGTGCTAT 450
E.multilocularis_Gr-Rodent          CTTGTTGTTGTTTCCATTGATTTATGGATTGTTTTTGGTGTGTGTGCTAT 439
E.multilocularis_Po-V.vulpes_7      CTTGTTGTTGTTTCCATTGATTTATGGATTGTTTTTGGTGTGTGTGCTAT 446
E.multilocularis_Es-V.vulpes        CTTGTTGTTGTTTCCATTGATTTATGGATTGTTTTTGGTGTGTGTGCTAT 418
E.multilocularis_Po-V.vulpes_7      CTTGTTGTTGTTTCCATTGATTTATGGATTGTTTTTGGTGTGTGTGCTAT 440
                                     **************************************************

E.multilocularis_Ak-V.lagopus*      GTGAGACTAATCGTATACCATTTGATTATGGGGAGTCT------ 488
E.multilocularis_Ch-Human           GTGAGACTAATCGTATACCATTTGATTATGGGGAGTCT------ 488
E.multilocularis_Gr-Rodent          GTGAGACTAATCGTACTCCATTTGATTATGGG------------ 471
E.multilocularis_Po-V.vulpes_7      GTGAGACTAATCGTATACCATTTGATTATGGGGAGTCT------ 484
E.multilocularis_Es-V.vulpes        GTGAGACT------------------------------------ 426
E.multilocularis_Po-V.vulpes_7      GTGAGACTAATCGTATACCATTTGATTATGGGGAGTCTGAAAGT 484
                                    ********
```

Figure 4.2 (Continued)


E.multilocularis Gr-Rodent
845
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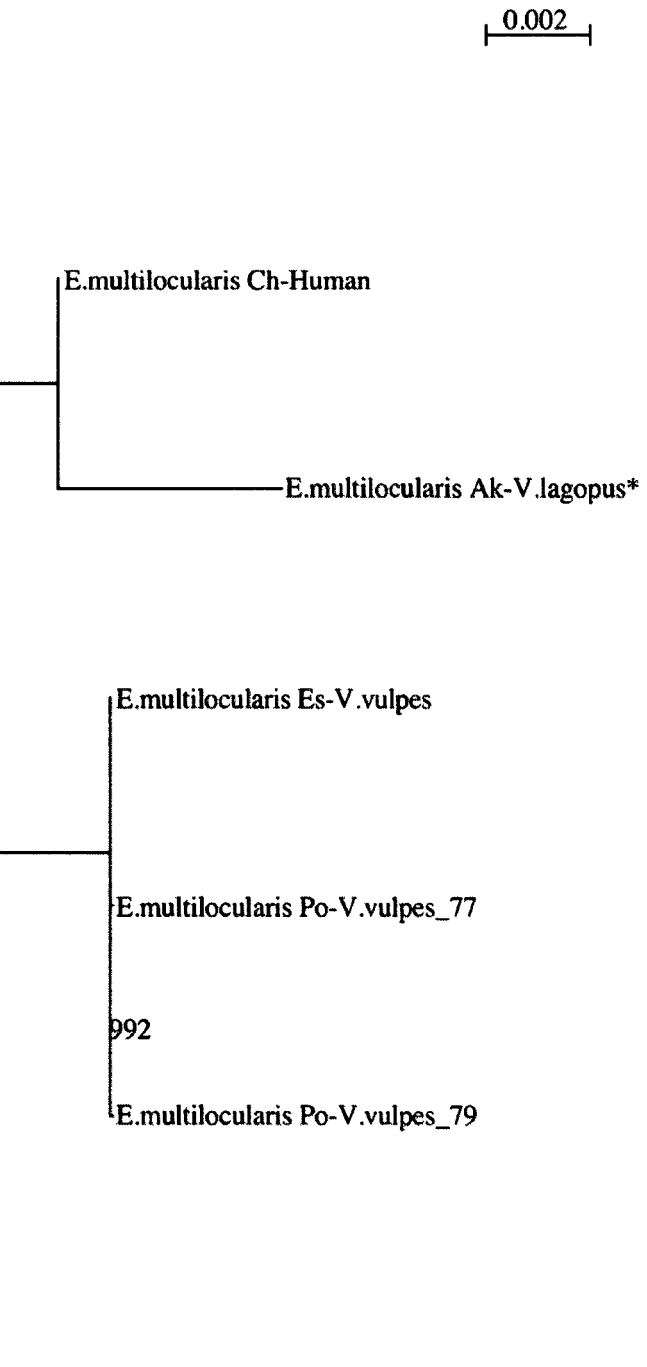
293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E.multilocularis Ch-Human
E.multilocularis Ak-V.lagopus*
E.multilocularis Es-V.vulpes
E.multilocularis Po-V.vulpes_77
992
E.multilocularis Po-V.vulpes_79

```
T.crassiceps_Sv-V.lagopus          AGGTGGGTATTATTGGGTTATTTCAGAGTTTTTCTGATTTACTAAAGTTA 50
T.crassiceps_Sv-M.levis            AGGTGGGTATTATTGGGTTATTTCAGAGTTTTTCTGATTTACTAAAGTTA 50
T.crassiceps_Ak-V.lagopus*         AGGTGGGTATTATTGGGTTATTTCAGAGTTTTTCTGATTTACTAAAGTTA 50
T.crassiceps_Ru-M.gregalis         AGGTGGGTATTATTGGGTTATTTCAGAGTTTTTCTGATTTACTAAAGTTA 50
T.crassiceps_Ak-M.pennsylvanic     AGGTGGGTATTATTGGGTTATTTCAGAGTTTTTCTGATTTACTAAAGTTA 50
T.crassiceps_Ru-M.fortis           AGGTGGGTATTATTGGATTATTTCAGAGTTTTTCTGATTTACTAAAGTTA 50
                                   ********** ********** ********** **********

T.crassiceps_Sv-V.lagopus          GTGGTTAAGTTTAAGAATTATAAATTTTGTGTACGTAGAAATTATAGTTT 100
T.crassiceps_Sv-M.levis            GTGGTTAAGTTTAAGAATTATAAATTTTGTGTACGTAGAAATTATAGTTT 100
T.crassiceps_Ak-V.lagopus*         GTGGTTAAGTTTAAGAATTATAAATTTTGTGTACGTAGAAATTATAGTTT 100
T.crassiceps_Ru-M.gregalis         GTGGTTAAGTTTAAGAATTATAAATTTTGTGTACGTAGAAATTATAGTTT 100
T.crassiceps_Ak-M.pennsylvanic     GTGGTTAAGTTTAAGAATTATAAATTTTGTGTACGTAGAAATTATAGTTT 100
T.crassiceps_Ru-M.fortis           GTGGTTAAGTTTAAGAATTATAAATTTTGTGTACGTAGAAATTATAGTTT 100
                                   **************************************************

T.crassiceps_Sv-V.lagopus          ATTAGGAGCTTTTCTTTTAGTTTTTTTAGTTATTTGTTATTCGTTTTTGT 150
T.crassiceps_Sv-M.levis            ATTAGGAGCTTTTCTTTTAGTTTTTTTAGTTATTTGTTATTCGTTTTTGT 150
T.crassiceps_Ak-V.lagopus*         ATTAGGAGCTTTTCTTTTAGTTTTTTTAGTTATTTGTTATTCGTTTTTGT 150
T.crassiceps_Ru-M.gregalis         ATTAGGAGCTTTTCTTTTAGTTTTTTTAGTTATTTGTTATTCGTTTTTGT 150
T.crassiceps_Ak-M.pennsylvanic     ATTAGGAGCTTTTCTTTTAGTTTTTTTAGTTATTTGTTATTCGTTTTTGT 150
T.crassiceps_Ru-M.fortis           ATTAGGAGCTTTTCTTTTAGTTTTTTTAGTTATTTGTTATTCGTTTTTGT 150
                                   **************************************************

T.crassiceps_Sv-V.lagopus          ATGGTGGTTATTATAGATTTAAATTTAAAAGTTTTTCTATGTTGTGAGTG 200
T.crassiceps_Sv-M.levis            ATGGTGGTTATTATAGATTTAAATTTAAAAGTTTTTCTATGTTGTGAGTG 200
T.crassiceps_Ak-V.lagopus*         ATGGTGGTTATTATAGATTTAAATTTAAAAGTTTTTCTATGTTGTGAGTG 200
T.crassiceps_Ru-M.gregalis         ATGGTGGTTATTATAGATTTAAATTTAAAAGTTTTTCTATGTTGTGAGTG 200
T.crassiceps_Ak-M.pennsylvanic     ATGGTGGTTATTATAGATTTAAATTTAAAAGTTTTTCTATGTTGTGAGTG 200
T.crassiceps_Ru-M.fortis           ATGGTGGTTATTATAGATTTAAATTTAAAAGTTTTTCTATGTTGTGAGTG 200
                                   **************************************************

T.crassiceps_Sv-V.lagopus          TTAGTTTTATCTAGTCTTTGTAGTTATTCTATTTTATGTGTGGGTTGAGG 250
T.crassiceps_Sv-M.levis            TTAGTTTTATCTAGTCTTTGTAGTTATTCTATTTTATGTGTGGGTTGAGG 250
T.crassiceps_Ak-V.lagopus*         TTAGTTTTATCTAGTCTTTGTAGTTATTCTATTTTATGTGTGGGTTGAGG 250
T.crassiceps_Ru-M.gregalis         TTGGTTTTATCTAGTCTTTGTAGTTATTCTGTTTTATGTGTGGGTTGAGG 250
T.crassiceps_Ak-M.pennsylvanic     TTAGTTTTATCTAGTCTTTGTAGTTATTCTATTTTATGTGTGGGTTGAGG 250
T.crassiceps_Ru-M.fortis           TTAGTTTTATCTAGTCTTTGTAGTTATTCTATTTTATGTGTGGGTTGAGG 250
                                   ** ************************** ********** **********
```

Figure 4.4 Clustal W alignment for partial nad1 gene sequence of *T crassiceps* isolated from arctic foxes and sled dogs in 1999, 2005, and 2007, northern Alaska

```
T.crassiceps_Sv-V.lagopus          TAGGTATAGTAAATATTCTTTTTTAAGTTCTGTTCGTTGTGCTTTTAGTT 300
T.crassiceps_Sv-M.levis            TAGGTATAGTAAATATTCTTTTTTAAGTTCTGTTCGTTGTGCTTTTAGTT 300
T.crassiceps_Ak-V.lagopus*         TAGSTATAGTAAATATTCTTTTTTAAGTTCTGTTCGTTGTGCTTTTAGTT 300
T.crassiceps_Ru-M.gregalis         TAGATATAGTAAATATTCTTTTTTAAGTTCTGTTCGTTGTGCTTTTAGTT 300
T.crassiceps_Ak-M.pennsylvanic     TAGATATAGTAAATATTCTTTTTTAAGTTCTGTTCGTTGTGCTTTTAGTT 300
T.crassiceps_Ru-M.fortis           TAGATATAGTAAATATTCTTTTTTAAGTTCTGTTCGTTGTGCTTTTAGTT 300
                                   *** ******************************************

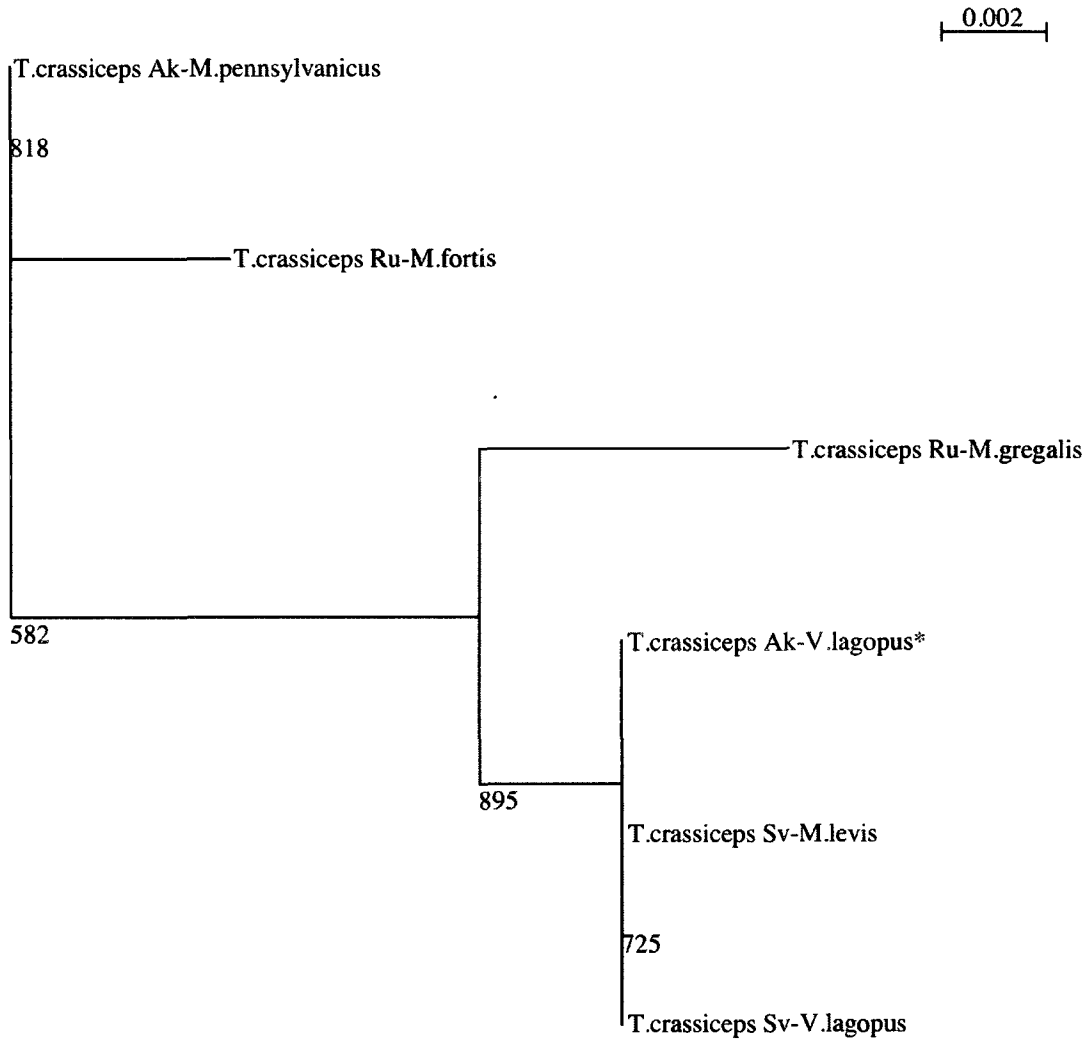
T.crassiceps_Sv-V.lagopus          CGATTAGGTTTGAAGCTTGTTTTATGTGTATTGTTGTATTTTCTGCGTTA 350
T.crassiceps_Sv-M.levis            CGATTAGGTTTGAAGCTTGTTTTATGTGTATTGTTGTATTTTCTGCGTTA 350
T.crassiceps_Ak-V.lagopus*         CGATTAGGTTTGAAGCTTGTTTTATGTGTATTGTTGTATTTTCTGCGTTA 350
T.crassiceps_Ru-M.gregalis         CGATTAGGTTTGAAGCTTGTTTTATGTGTATTGTTGTGTTTTCTGCGTTA 350
T.crassiceps_Ak-M.pennsylvanic     CGATTAGGTTTGAAGCTTGTTTTATGTGTATTGTTGTTTTTTCTGCGTTA 350
T.crassiceps_Ru-M.fortis           CGATTAGGTTTGAAGCTTGTTTTATGTGTATTGTTGTTTTTTCTGCGTTA 350
                                   ************************************ ************

T.crassiceps_Sv-V.lagopus          TGTTTTAGTAGTTATAGTTTGGTAGATGTGTATTTTAGGGATTGATCATT 400
T.crassiceps_Sv-M.levis            TGTTTTAGTAGTTATAGTTTGGTAGATGTGTATTTTAGGGATTGATCATT 400
T.crassiceps_Ak-V.lagopus*         TGTTTTAGTAGTTATAGTTTGGTAGATGTGTATTTTAGGGATTGATCATT 400
T.crassiceps_Ru-M.gregalis         TGTTTTAGTAGTTATAGTTTGGTAGATGTGTATTTTAGGGATTGATCATT 400
T.crassiceps_Ak-M.pennsylvanic     TGTTTTAGTAGTTATAGTTTGGTGGATGTGTATTATAGGGATTGATCATT 400
T.crassiceps_Ru-M.fortis           TGTTTTAGTAGTTATAGTTTGGTGGATGTGTATTATAGGGATTGATCATT 400
                                   ********************** ********** ***************

T.crassiceps_Sv-V.lagopus          GATTTTGTTGTTTCCGTTAATTATAATTGTGTATTTAGTATGTATTTTGT 450
T.crassiceps_Sv-M.levis            GATTTTGTTGTTTCCGTTAATTATAATTGTGTATTTAGTATGTATTTTGT 450
T.crassiceps_Ak-V.lagopus*         GATTTTGTTGTTTCCGTTAATTATAATTGTGTATTTAGTATGTATTTTGT 450
T.crassiceps_Ru-M.gregalis         GATTTTGTTGTTTCCGTTAATTATAATTGTGTATTTAGTATGTATTTTGT 450
T.crassiceps_Ak-M.pennsylvanic     GATTTTGTTGTTTCCGTTAATTATAATTGTGTACTTAGTATGTATTTTGT 450
T.crassiceps_Ru-M.fortis           GATTTTGTTGTTTCCGTTAATTATAATCGTGTACTTAGTATGTATTTTGT 450
                                   ************************** ***** ***************

T.crassiceps_Sv-V.lagopus          GTGAAACGAAACGTAGTCCATTTGATTATGGGGAGGCT 488
T.crassiceps_Sv-M.levis            GTGAAACGAAACGTAGTCCATTTGATTATGGGGAGGCT 488
T.crassiceps_Ak-V.lagopus*         GTGAAACGAAACGTAGTCCATTTGATTATGGGGAGGCT 488
T.crassiceps_Ru-M.gregalis         GTGAAACGAAACGTAGTCCATTTGATTATGGGGAGGCT 488
T.crassiceps_Ak-M.pennsylvanic     GTGAAACGAAACGTAGTCCATTTGATTATGGGGAGGCT 488
T.crassiceps_Ru-M.fortis           GTGAAACGAAACGTAGTCCATTTGATTATGGGGAGGCT 488
                                   ************************************
```

Figure 4.4 (Continued)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T.crassiceps Ak-M.pennsylvanicus
818
T.crassiceps Ru-M.fortis
T.crassiceps Ru-M.gregalis
582
T.crassiceps Ak-V.lagopus*
895
T.crassiceps Sv-M.levis
725
T.crassiceps Sv-V.lagopus

```
T.polycantha_De-M.glaroelus       AGGTTGGTATAATGGGTTTATTTCAAAGTTTTTCTGATTTGTTGAAGTTA 50
T.polycantha_Sc-M.glaroelus       AGGTTGGTATAATGGGTTTATTTCAAAGTTTTTCTGATTTGTTGAAGTTA 50
T.polycantha_Fi-V.lagopus         AGGTTGGTATAATGGGTTTATTTCAAAGTTTTTCTGATTTGTTGAAGTTA 50
T.polycantha_Tu-M.guentheri       AGGTTGGTATAATGGGTTTATTTCAAAGTTTTTCTGATTTGTTGAAGTTA 50
T.polycantha_Ak-V.lagopus*        AGGTTGGTATAATGGGTTTGTTTCAAAGTTTTTCTGATTTGTTGAAGTTG 50
T.polycantha_Sv-V.lagopus         AGGTTGGTATAATGGGTTTGTTTCAAAGTTTTTCTGATTTGTTGAAGTTG 50
T.polycantha_Ca-L.trimucronatu    AGGTTGGTATAATGGGTTTGTTTCAAAGTTTTTCTGATTTGTTGAAGTTG 50
T.polycantha_Gr-D._groenlandic    AGGTTGGTATAATGGGTTTGTTTCAAAGTTTTTCTGATTTGTTGAAGTTG 50
                                  ******************.****************************.

T.polycantha_De-M.glaroelus       ATAGTTAAGAGTAAGGAATATTATTTTAGTAGTCGTAGTTGGGTAGGTTT 100
T.polycantha_Sc-M.glaroelus       ATAGTTAAGAGTAAGGAATATTATTTTAGTAGTCGTAGTTGGGTAGGTTT 100
T.polycantha_Fi-V.lagopus         ATAGTTAAGAGTAAGGAATATTATTTTAGTAGTCGTAGTTGGGTAGGTTT 100
T.polycantha_Tu-M.guentheri       ATAGTTAAGAGTAAGGAATATTATTTTAGTAGTCGTAGTTGGGTAAGTTT 100
T.polycantha_Ak-V.lagopus*        ATAATTAAGAGTAAGAGTTTTTATTTTAGGAGTCGTAGATGAGTGGGTTT 100
T.polycantha_Sv-V.lagopus         ATAATTAAGAGTAAGAGTTTTTATTTTAGTAGTCGTAGATGAGTGGGTTT 100
T.polycantha_Ca-L.trimucronatu    ATAATTAAGAGTAAGAGTTTTTATTTTAGTAGTCGTAGATGAGTGGGTTT 100
T.polycantha_Gr-D._groenlandic    ATAATTAAGGGTAAGAATTTTTATTTTAGGAGTCGTAGATGAGTGGGTTT 100
                                  ***.*****.*****..:*:********* ********:**.**..****

T.polycantha_De-M.glaroelus       GTTTGGGGTTTTTTTATTGGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Sc-M.glaroelus       GTTTGGGGTTTTTTTATTGGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Fi-V.lagopus         GTTTGGGGTTTTTTTATTGGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Tu-M.guentheri       GTTTGGGGTTTTTTTATTGGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Ak-V.lagopus*        ATTTGGAGTTTTTTTATTAGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Sv-V.lagopus         ATTTGGAGTTTTTTTATTAGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Ca-L.trimucronatu    ATTTGGAGTTTTTTTATTAGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
T.polycantha_Gr-D._groenlandic    ATTCGGAGTTTTTTTATTAGTTTGTTTGGTTTTGTTTTATTCATTTATAT 150
                                  .** **.************.******************************

T.polycantha_De-M.glaroelus       ATGGTGGATATTATAGGTCTAGTTTTAATTCTCTTTCTTTATTGTGATTT 200
T.polycantha_Sc-M.glaroelus       ATGGTGGATATTATAGGTCTAGTTTTAATTCTCTTTCTTTATTGTGATTT 200
T.polycantha_Fi-V.lagopus         ATGGTGGATATTATAGGTCTAGTTTTAATTCTCTTTCTTTATTGTGATTT 200
T.polycantha_Tu-M.guentheri       ATGGTGGATATTATAGGTCTAGTTTTAATTCTCTTTCTTTATTGTGGTTT 200
T.polycantha_Ak-V.lagopus*        ATGGTGGGTATTATAGGTCTAGATTTAATTCTCTTTCTTTATTATGGTTT 200
T.polycantha_Sv-V.lagopus         ATGGTGGGTATTATAGGTCTAGATTTAATTCTCTTTCTTTATTATGGTTT 200
T.polycantha_Ca-L.trimucronatu    ATGGTGGGTATTATAGGTCTAGATTTAATTCTCTTTCTTTATTATGGTTT 200
T.polycantha_Gr-D._groenlandic    ATGGTGGGTATTATAGGTCTAGATTTAATTCTCTTTCTTTATTATGGTTT 200
                                  *******.***************:********************.**.***

T.polycantha_De-M.glaroelus       TTAGTGGTTTCAAGTTTTTGTAGGTATGCGATAATGTCTGCTGGTTGAGG 250
T.polycantha_Sc-M.glaroelus       TTAGTGGTTTCAAGTTTTTGTAGGTATGCGATAATGTCTGCTGGTTGAGG 250
T.polycantha_Fi-V.lagopus         TTAGTGGTTTCAAGTTTTTGTAGGTATGCGATAATGTCTGCTGGTTGAGG 250
T.polycantha_Tu-M.guentheri       TTAGTGGTTTCAAGTTTTTGTAGGTATGCGATAATGTCTGCTGGTTGAGG 250
T.polycantha_Ak-V.lagopus*        TTAGTTATTTCTAGTTTTTGTAGGTATGCCATAATGTCTGCTGGTTGAGG 250
T.polycantha_Sv-V.lagopus         TTAGTTATTTCTAGTTTTTGTAGGTATGCCATAATGTCTGCTGGTTGGGG 250
T.polycantha_Ca-L.trimucronatu    TTAGTTATTTCTAGTTTTTGTAGGTATGCCATAATGTCTGCTGGTTGAGG 250
T.polycantha_Gr-D._groenlandic    TTAGTCATTTCTAGTTTTTGTAGGTACGCCATAATGTCTGCTGGTTGAGG 250
                                  ***** .****:************** ** *****************.**
```

Figure 4.6 Clustal W alignment for partial nad1 gene sequence of *T. polyacantha* isolated from an arctic fox in 1999, northern Alaska.

```
T.polycantha_De-M.glaroelus         TAGTTATAGTAATTATTCATTCTTGAGTTCAATACGTTGTGCGTTTGGTT 300
T.polycantha_Sc-M.glaroelus         TAGTTATAGTAATTATTCATTCTTGAGTTCAATACGTTGTGCGTTTGGTT 300
T.polycantha_Fi-V.lagopus           TAGTTATAGTAATTATTCATTCTTGAGTTCAATACGTTGTGCGTTTGGTT 300
T.polycantha_Tu-M.guentheri         TAGTTATAGTAATTATTCATTCTTGAGTTCAATACGTTGTGCGTTTGGTT 300
T.polycantha_Ak-V.lagopus*          TAGTTATAGCAATTATTCATTTTTGAGTTCGATACGTTGTGCATTTGGTT 300
T.polycantha_Sv-V.lagopus           TAGTTATAGCAATTATTCATTTTTGAGTTCGATACGTTGTGCATTTGGTT 300
T.polycantha_Ca-L.trimucronatu      TAGTTATAGAAATTATTCATTTTTGAGTTCGATACGTTGTGCATTTGGTT 300
T.polycantha_Gr-D._groenlandic      TAGTTATAGTAATTATTCATTTTTGAGTTCGATACGTTGTGCGTTTGGTT 300
                                    ********* ********** ********.**********.*******

T.polycantha_De-M.glaroelus         CTATAAGATTTGAGGCATGTTTTATGTGTATTGTTATATTTTCTGTGTTA 350
T.polycantha_Sc-M.glaroelus         CTATAAGATTTGAGGCATGTTTTATGTGTATTGTTATATTTTCTGTGTTA 350
T.polycantha_Fi-V.lagopus           CTATAAGATTTGAGGCATGTTTTATGTGTATTGTTATATTTTCTGTGTTA 350
T.polycantha_Tu-M.guentheri         CTATAAGATTTGAGGCATGTTTTATGTGTATTGTTATATTTTCTGTGTTA 350
T.polycantha_Ak-V.lagopus*          CTATAAGATTTGAGGCATGTTTTATGTGCATAGTGATTTTTTCTGTGTTG 350
T.polycantha_Sv-V.lagopus           CTATAAGATTTGAGGCATGTTTTATGTGCATAGTGATTTTTTCTGTGTTG 350
T.polycantha_Ca-L.trimucronatu      CTATAAGATTTGAGGCATGTTTTATGTGCATAGTGATTTTTTCTGTGTTG 350
T.polycantha_Gr-D._groenlandic      CTATAAGATTTGAGGCATGTTTTATGTGTATAGTGATTTTTTCTGTGTTG 350
                                    *************************** **:** **:***********.

T.polycantha_De-M.glaroelus         TGTTGTGGTAGGTATAGTTTGTTGGATTTTTTTTATTTAGATTATTTTTC 400
T.polycantha_Sc-M.glaroelus         TGTTGTGGTAGGTATAGTTTGTTGGATTTTTTTTATTTAGATTATTTTTC 400
T.polycantha_Fi-V.lagopus           TGTTGTGGTAGGTATAGTTTGTTGGATTTTTTTTATTTAGATTATTTTTC 400
T.polycantha_Tu-M.guentheri         TGTTGTGGTAGGTATAGTTTGTTGGATTTTTTTTATTTAGATTATTTTTC 400
T.polycantha_Ak-V.lagopus*          TGTTATGGTAGTTATAGTTTGTTAGATTATTTTTATTTAGATTATTTTTC 400
T.polycantha_Sv-V.lagopus           TGTTATGGTAGTTATAGTTTGTTAGATTATTTTTATTTAGATTATTTTTC 400
T.polycantha_Ca-L.trimucronatu      TGTTATGGTAGTTATAGTTTGTTAGATTATTTTTATTTAGATTATTTTTC 400
T.polycantha_Gr-D._groenlandic      TGTTATGGTAGTTATAGTTTGTTAGATTATTTTTATTTAGATTATTTTTC 400
                                    ****.****** **********.****:********************

T.polycantha_De-M.glaroelus         TTGTATTATATACCCATGTGTATTTATATTGTATTTGATTTGTATATTAT 450
T.polycantha_Sc-M.glaroelus         TTGTATTATATACCCATGTGTATTTATATTGTATTTGATTTGTATATTAT 450
T.polycantha_Fi-V.lagopus           TTGTATTATATACCCATGTGTATTTATATTGTATTTGATTTGTATATTAT 450
T.polycantha_Tu-M.guentheri         TTGTATTATATACCCATGTGTATTTATATTGTATTTGATTTGTATATTAT 450
T.polycantha_Ak-V.lagopus*          TTGTATCATATACCCATGTATCTTTGTATTGTATCTTGTTTGTATATTAT 450
T.polycantha_Sv-V.lagopus           TTGTATCATATACCCATGTATCTTTGTATTGTATCTTGTTTGTATATTAT 450
T.polycantha_Ca-L.trimucronatu      TTGTATCATATACCCATGTATCTTTGTATTGTATCTTGTTTGTATATTAT 450
T.polycantha_Gr-D._groenlandic      TTGTATTATATATCCATGTATCTTTATATTGTATCTTGTTTGTATATTAT 450
                                    ****** ***** ******.*.***.******** * .************

T.polycantha_De-M.glaroelus         GTGAGACTAATCGTATTCCATTTGATTATAGTGAGGCT 488
T.polycantha_Sc-M.glaroelus         GTGAGACTAATCGTATTCCATTTGATTATAGTGAGGCT 488
T.polycantha_Fi-V.lagopus           GTGAGACTAATCGTATTCCATTTGATTATAGTGAGGCT 488
T.polycantha_Tu-M.guentheri         GTGAGACTAATCGTATTCCATTTGATTATA-------- 480
T.polycantha_Ak-V.lagopus*          GTGAGACTAATCGTACCCCATTTGATTATAGAGAATCT 488
T.polycantha_Sv-V.lagopus           GTGAGACTAATCGTACCCCATTTGATTATAGAGAATCT 488
T.polycantha_Ca-L.trimucronatu      GTGAGACTAATCGTACCCCATTTGATTATAGAGAATCT 488
T.polycantha_Gr-D._groenlandic      GCGAGACTAATCGTACCCCGTTTGATTATAGAGAGTCT 488
                                    * *************  **.**********
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Figure 4.6 (Continued)

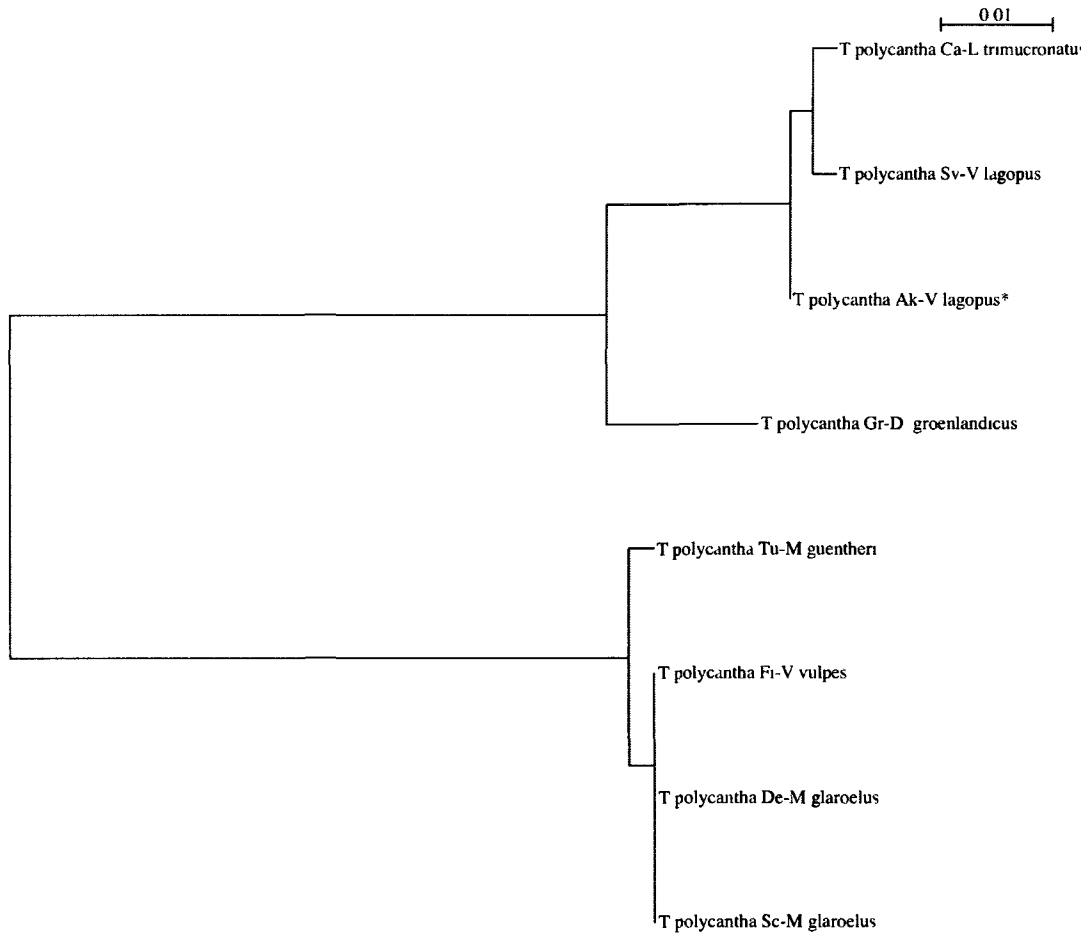
0 01
T polycantha Ca-L trimucronatus
T polycantha Sv-V lagopus
T polycantha Ak-V lagopus*
T polycantha Gr-D groenlandicus
T polycantha Tu-M guentheri
T polycantha Fi-V vulpes
T polycantha De-M glaroelus
T polycantha Sc-M glaroelus

Table 4.1 Prevalence of Taeniidae in arctic fox and sled dogs for three sampling years in northern Alaska.

(a)

Taeniidae Prevalence in Arctic Fox

			Year (N)					
	Total (46)		**1999 (26*)**		**2005 (8♦)**		**2007 (12♦)**	
Species	**Prevalence**	**95% CI**	**Prevalence**	**95% CI**	**Prevalence**	**95% CI**	**Prevalence**	**95% CI**
E. multilocularis	15.20%	6.3-28.9%	26.90%	11.6-47.8%	0%	0-36.9%	0%	0-26.5%
T. crassiceps	47.80%	32.9-63.1%	42.30%	23.4-63.1%	50%	15.7-84.3%	8.30%	0.2-38.5
T. polyacantha	2.20%	0.1-11.5%	2.20%	0.1-11.5%	0%	0-36.9%	0%	0-26.5%

(b)

Taeniidae Prevalence in Sled dogs

	2007 (12♦)	
Species	**Prevalence**	**95% CI**
E. multilocularis	0%	0-26.5%
T. crassiceps	58.30%	27.8-84.8%
Mixed/Equivocal	41.70%	15.2-72.3%

*Samples collected from the cecum of necropsied animals.

♦Samples were defecated from live animals (fecals).

CONCLUSION

Investigators anticipate that the health of Arctic marine ecosystems will be disrupted by climate change and the associated loss of sea ice (ACIA, 2004; Fuglei and Ims, 2008; Regehr et al., 2009; Stirling et al., 1999). The status of polar bears and arctic fox as arctic sentinels facilitates their use to assess climate change effects on ecosystem health. By monitoring the health status and exposure to infectious agents of these sentinels, we can gain insight into the impact of ongoing changes in the Arctic. To this end, this thesis strives to coalesce the relevant biological variables and ecological context in the analysis and interpretation of these data.

Currently, limited data exist on the health status of U.S. polar bear populations. We provide a benchmark for hematological parameters of southern Beaufort Sea polar bears, thus facilitating assessment of this biomarker over time. We also establish seroprevalence and risk factors for "indicator" pathogens in this species. Associations we develop between serological evidence of exposure to infectious agents and hematological parameters described substantiate the value of using this biomarker to monitor polar bear health. These measures provide a "tool set" for evaluating Arctic ecosystem health.

Lowered counts of some leukocyte types in female polar bears with dependent young suggest this cohort may be less resilient than other sex and age groups to new challenges that may accompany ongoing ecosystem changes. Lowered resilience of reproducing

females may compound the already observed declines in reproductive performance of females related to declining sea-ice availability (Stirling et al., 1999; Regehr et al., 2009). This cohort is directly responsible for recruitment and thus is also important for status and trends assessment. Therefore, this cohort should be targeted in future polar bear health monitoring, research programs, and conservation monitoring that assesses arctic ecosystem health.

A warming Arctic may alter microbial and parasite transmission pathways as well as susceptibility of apex predators, including humans, to disease. However, we lack basic information on the current ecology of most infectious agents in northern Alaska. Because morbilliviruses and *T. gondii* pass between terrestrial and aquatic environments, they are of concern for marine organisms in a warming world. Changes in morbillivirus and *T. gondii* prevalence that may occur with climate change could pose an increased threat to the health of local people living along the coast and wildlife.

We examined a fragment of the P gene in morbillivirus isolated from arctic fox during and outbreak of rabies and during 2007. The morbillivirus appears canine (terrestrial) at the genetic level however; it cannot be distinguished from marine strains using diagnostic serology assays. Although we were unable to examine viral sequences from polar bears, serology suggests morbillivirus circulating among polar bears is distinct from that which infected sympatric arctic foxes in 2007. Without viral RNA sequences from polar bears, seals, or other sympatric species of arctic fox, we cannot determine which cross species

transmissions of the virus are significant in northern Alaska. The results of this study underscore the limitations of using serology alone to examine morbillivirus epizootiology. Phylogenetic analyses however, do suggest that the strain isolated from arctic fox is distinct from European, Asian, Eurasian, and North American field strains and most closely related to "arctic isolates" of CDV. Future studies should target isolation of morbilliviral nucleic acid from other carnivores across the Arctic and also sequence the less conserved H and fusion (F) genes of the strain isolated from North Slope arctic fox, in order to elucidate the phylogeny of this virus in the Arctic. Isolation of species and region specific pathogens is very important for development of appropriate research and diagnostic tools. Thus we provide a practical means to fulfill this need in the context of the "One Health" perspective.

The hematology and serology data we present for Alaskan polar bears emphasize the potential importance of synergisms among various potential stressors (morbillivirus, organochlorines, climate change, increased human presence etc.) that may undermine the resistence (immune system) of polar bears to changes in their habitats. We have set the stage to use these hematologic measures as a biomarker to assess polar bear responses to changing infectious agents, nutritional status, and toxicant exposure that may result from global warming. The seroprevalence patterns we establish for two "indicator" infectious agents (morbillivirus and *T. gondii*) and associations we demonstrate between seroprevalence and hematological biomarkers illustrate how monitoring polar bear health can provide an index of changing pathogen occurrence throughout the Arctic. Similarly,

we propose canids to be the best sentinels for monitoring the impact of climate change on the ecology of *Echinococcus*.

Despite its public health significance as a zoonosis, there is limited information available regarding the current ecology and transmission dynamics of *E. multilocularis* in the northern Alaska. With anticipated changes in Arctic ecology due to climate change and increasing anthropogenic influences, prevalence of this zoonotic agent is likely to change with host range shifts. Understanding its ecology and monitoring its prevalence will be necessary to understand the human health consequences of ongoing and projected changes in the Arctic. We identified the species of *Echinococcus* present in arctic fox on the Alaska North Slope as *E. multilocularis*. Similar to our investigation of CDV, such studies can be conducted opportunistically as samples become available from rabies and predator control programs, trapping and health assessments of Arctic canids. Although we could not assess the relationship of the strain we isolated from arctic fox to North America strains (genetic information was unavailable in the database), the North Slope arctic fox isolate was most similar to Asian isolates based on maximum likelihood analysis. This observation is consistent with the previous detection of Asian isolates in St. Lawrence Island, Alaska. Sequence analysis of isolates of *T. crassiceps* and *T. polyacantha* demonstrated the greatest homology with isolates derived from the same host (*V. lagopus*).

Ongoing and anticipated changes in the Arctic have generated concern for both the health

of human and of wildlife populations and communities. The health of ecosystems is reflected in the health of animals inhabiting those ecosystems and the identification of Arctic sentinels is critical at this time, and possibly overdue. Many data gaps remain regarding the status of Arctic fauna, which hinder our ability to assess the impacts of climate change on ecosystem health in the Arctic. Baseline health information on monitored species is needed in order to address change over time and to better interpret data collected on movement (e.g., satellite collars), body condition (morphometrics), abundance and trends (mark-recapture), and feeding ecology. Furthermore, there is a need to improve knowledge of pathogens currently present, as well at their transmission dynamics and to establish mechanisms to detect emergence of pathogens of concern. With the use of biomarkers, we may detect changes at the physiological level before they manifest at the population level, thus facilitating proactive measures with a greater chance of success (noting a significant change in abundance or recruitment is "after the fact"). We suggest that a "One Health" approach provides the best tools for addressing these knowledge gaps and provide examples of how this may be accomplished in the Arctic. Understanding Arctic ecosystem health will require the collaborative efforts of experts in diverse fields as well as input from local, traditional ecological knowledge over the proper spatial and temporal scales.

REFERENCES

ACIA. 2004. Impacts of a warming Arctic. Arctic Climate Impact Assessment. Cambridge, United Kingdom: Cambridge University Press, 144 pp.

Fuglei E, Ims RA. 2008. Global warming and effects on the arctic fox. Science Progress 91(2):175-191.

Regehr EV, Hunter CM, Caswell H, Amstrup SC, Stirling I. 2009. Survival and breeding of polar bears in the southern Beaufort Sea in relation to sea ice. Journal of Animal Ecology doi: 10.1111/j.1365-2656.2009.01603.x [Online September 14, 2009].

Stirling I, Lunn NJ, Iacozza J. 1999. Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. Arctic (3)52:294-306.